GENERAL PROTOCOLS
FOR A
JOINT UKRAINIAN AMERICAN FOLLOW-UP OF RADIATION CATARACTS IN UKRAINIANS INVOLVED IN THE CHERNOBYL ACCIDENT

Agreed to:

by

The Ukrainian-American Chernobyl Ocular Study (UACOS) Group

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In accordance with our joint Letter of Intent dated April 13, 1992, we the signatories agree on the enclosed protocols and organizational structure this day, April 15, 1993.

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INTRODUCTION

In the early morning of April 26, 1986, reactor number four of the Chernobyl nuclear power complex underwent a power excursion resulting in a steam explosion which spewed solid and gaseous radioactive materials into the environment. From the initial explosion and during the subsequent hours to days, secondary releases, some as a direct result of an attempt to deal with the exposed core, caused a large civilian population to receive significant doses. The entire city of Pripyat (48,000 people) was evacuated 36 hours after the initial explosion, and a total of 116,500 people within a 30 km radius of the blast was relocated (Omelyanets et al., 1988). Together about 600,000 people were exposed to radiation from the accident. Those who were assigned the clean-up and maintenance duties in the months and years following the disaster offer a unique laboratory for the study of human radiation exposure at the individual and the populational level. The workers, conscriptees, and volunteer army, who comprised the so-called "**liquidators**", numbered in excess of 200,000. Every effort has been made to determine the exposure level and fully define the population involved. A registry, "The National Registry of the Ministry of Health", has been established which is continually being updated. It contains 130,000 1986-1987 liquidators with a mean exposure of about 0.15 Sv. Up to 15% received >0.3 Sv and ~2% were exposed to doses greater than 0.7 Sv. While of little comfort to the victims, this tragic experience has produced a laboratory and a reference population which can be used to better understand radiation effects in the human population and develop the means to assess them. We hope to achieve both in the present collaboration by an intensive focus on the eye, with particular emphasis on the lens and its classic radiogenic pathology, cataract.

That cataracts arise from exposure to ionizing radiation is a fact which has historically been well appreciated by those involved in risk assessment and safety determinations. The lens, by virtue of its primary pathology, cataract, has enjoyed a long history as a sort of biological dosimeter. The first attempt at true calibration of this monitoring technique was done by Merriam and Focht in 1962 using a limited population of radiotherapy patients. Although relatively few low dose exposures were available (the total population numbered under 300 with 34 cases receiving less than 2 Gy) that work, suggested a threshold for cataract of 2 Gy. The most recent ophthalmic reevaluation of the Hiroshima data base based on DS86 criteria suggests a minimum cataractogenic dose of about 0.7 Sv. A recent study of an American population (Beaver Dam, Wisconsin) (Klein et al., 1993) indicates a higher prevalence of posterior subcapsular cataracts in patients who had received CAT scans, suggesting that doses on the order of 10 to 30 cGy are cataractogenic. A recent report on ocular changes in children from exposed regions around Chernobyl indicate a higher than normal incidence of posterior subcapsular changes (Eller et al., 1993). While the precise dose range cannot be ascribed to the exposed children it is very unlikely that it exceeded tens of cSv with a high probability than most of the children were exposed to a fraction of that. These data are consonant with experimental evidence (Worgul et al., 1993) suggesting the absence of a threshold for cataracts and that the cataractogenic response to radiation is purely stochastic.

In terms of cataract in the Chernobyl population, it is becoming apparent that we are at the point when opacities are beginning to develop. The problem is that while radiation cataracts develop in a characteristic fashion, the changes are not pathognomonic. The clinical picture requires consideration of onset time, progression, and a robust personal history. It is important that a non-subjective, permanent record be made of a subgroup developing lens changes. Because it is clear that the population is available, ready to study, and the post-accident time is such that cataracts may be beginning to appear, we cannot afford to delay the initiation of an ophthalmological follow-up on the liquidator population. This group represents the most dosimetrically defined of the Chernobyl population. Also, because their exposure was occupationally related, they are continuously monitored and readily available for longitudinal analyses. The situation provides an unparalleled opportunity to not only consider risk issues, but modalities for monitoring populations at risk and to test existing theories of radiation action on normal tissues in general, and the eye in particular.

OBJECTIVES

The ocular radiation protection standards formulated by national and international committees are all predicated on the assumption that there exists a high dose threshold for radiation cataract development. If, as the evidence is increasingly suggesting, this supposition is incorrect, the standards set for radiation workers as well as the population at large may not be appropriate. Clearly, in order to adequately protect those at risk of radiation exposure this question must be resolved.

The Chernobyl population provides, by its size, dose distribution and accessibility, the first real opportunity to address the question of whether or not radiation cataracts are deterministic or stochastic. For the same reasons it can also supply the data necessary to allow radiation cataract acheive its full potential as a "biological dosimeter" for assessing populations at risk and reducing uncertainties in retospective dose reconstruction.

The planned joint Ukrainian/American investigations are designed to apply state-of-the-art methodologies and technologies to assess damage to the lenses of individuals exposed to radiation resulting from the Chernobyl Reactor Number Four accident. The data is meant not only to document the ocular health of the exposed population, but to derive therefrom a greater resolution of our understanding of the cataractogenic effects of radiation exposure in human populations, in order to maximize its potential for retrospective dosimetry. The project also includes a tissue rescue program (repository) for bioindicator calibration and future cytologic reference.

The effort will focus on the dose and time dependent effects, primarily among the liquidator population and will include, although not be limited to, the following:

1. A cohort epidemiological study (with a nested case control subset) of cataract onset and progression using standardized subjective parameters.
2. Quantitative analyses of radiation cataract development and progression in humans employing new technologies for a longitudinal non-subjective evaluation of lens transparency.
3. The establishment of a program for the acquisition, archiving and analyses of lens epithelial tissue removed during routine cataract extraction procedures.

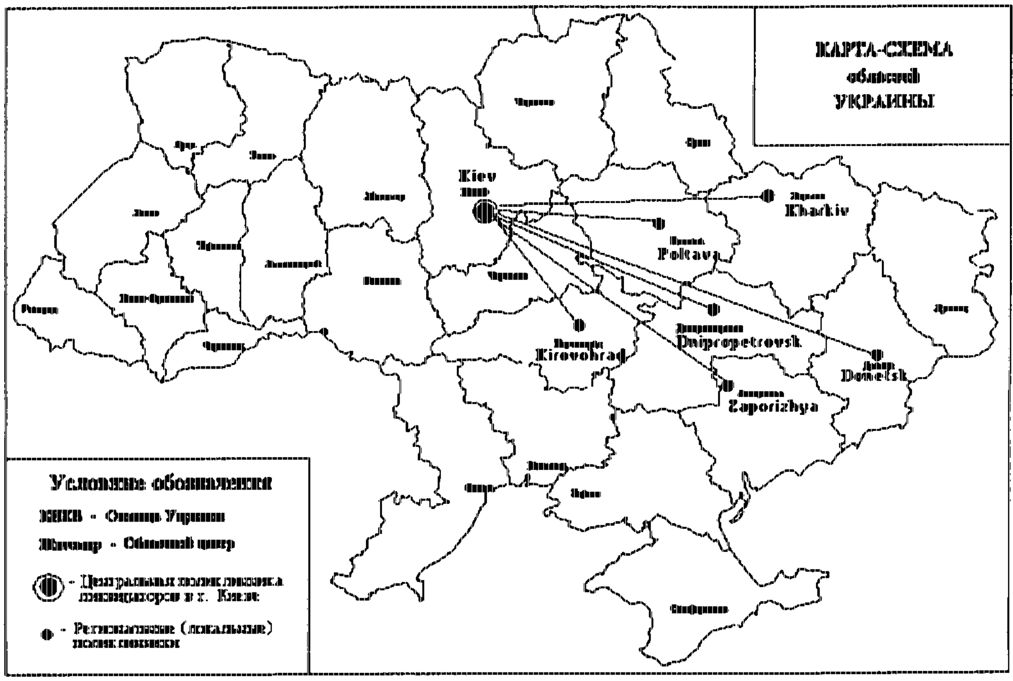
These studies will be accompanied by a thorough ocular examination as well as an epidemiological evaluation of other exposures to establish potentially confounding factors which may influence the outcome. Ultimately we hope to achieve the following **Specific Aims**:

1. **To obtain a reliable evaluation of the risk of cataract development in a critical population involved in the Chernobyl accident.**
2. **To non-subjectively measure and quantify human radiation cataract development as a function of dose and establish a reference database which can be used to retrospectively assess radiation exposure in individuals.**
3. **Calibrate the micronucleus assay in the lens as a potential retrospective dosimetric bioindicator for evaluating populations at risk and establish a repository of tissue for future reference.**

To avoid the oversights of past population studies that were later found to require reanalyses every effort will be made to archive the raw information in a manner which would allow future retrospective reassessment. Obviously Specific aims 2 and 3 are ideally suited to this end and are designed for the possibility of later access. A goal of Specific Aim 2 is to produce an digitized image library of cataract development following exposure to a variety of doses. Specific Aim 3 will establish a tissue "bank" of processed and unprocessed epithelia from Chernobyl exposed individuals. Although Specific Aim 1 is a classic cohort study (with a nested case control analysis) it too will require slitlamp photographic documentation of a large fraction of those included in the study. The pictures which are to be taken using a standardized format will be catalogued and made available for later examination.

ORGANIZATIONAL STRUCTURE

Aim 1: Aim 1 is to conduct a longitudinal study of cataract prevalence and incidence in a cohort of radiation-exposed Chernobyl liquidators (i.e., clean-up workers) with the following objectives. 1) To determine whether there is evidence that the induction of by radiation is a stochastic, rather than deterministic, process. This implies that there would be excess risk at lower doses, rather than a dose threshold. This involves examining the dose-response relationship below about 70 cGy (which was the dose threshold estimated by the investigators of the A-bomb survivor data). 2) To determine the risk of radiation-induced posterior subcapsular cataracts (PSCs) among liquidators exposed to relatively high lens doses (say, >70 cGy). This question needs to be examined because the exposures range from acute to protracted, unlike the A-bomb survivor study (Otake and Schull, 1990) and the medical irradiation studies (Merriam and Focht, 1957). 3) A secondary objective is to examine ultraviolet radiation exposure, smoking, alcohol consumption, diet (vitamins A and C, carotenoids), certain medicinal drugs and occupational genotoxic agents as co-factors in cataract risk. The studies will be integrated into a fully inclusive ophthalmic screening (preventative mediums) program provided by the Ministry of Health, Ukraine.

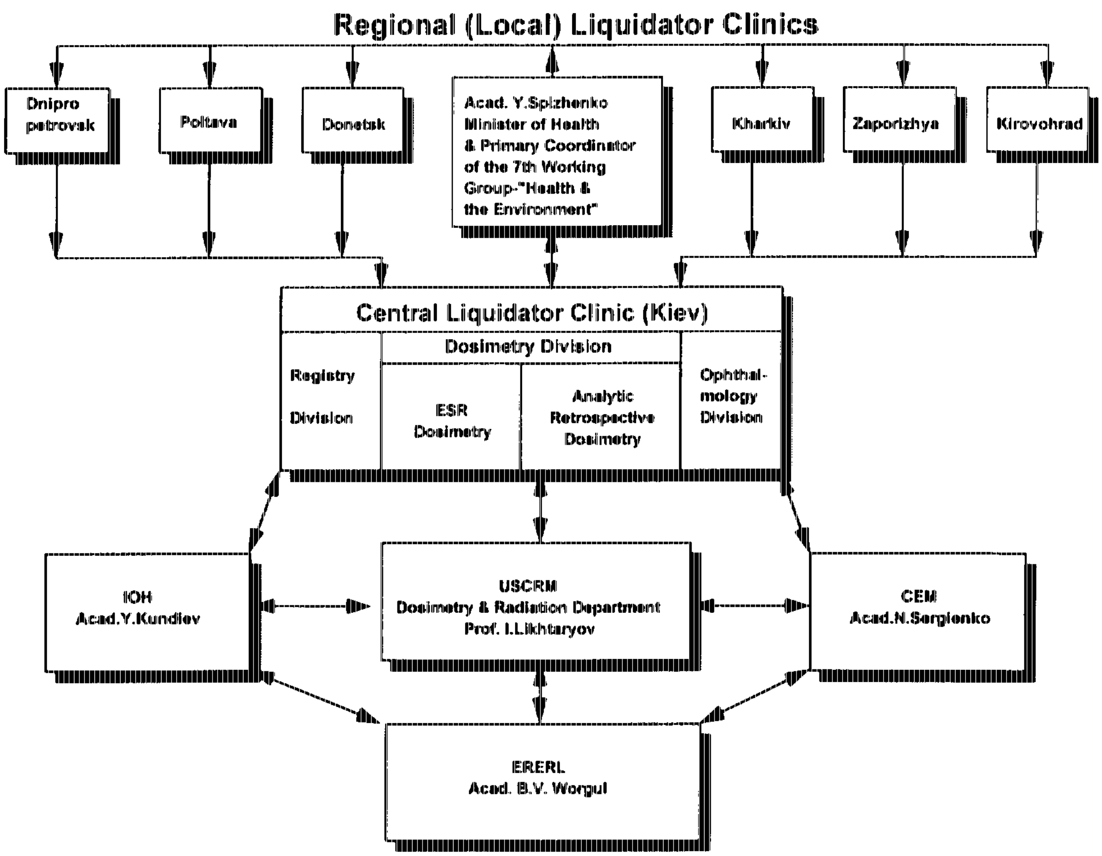


This protocol is designed to be compatible with existing arrangements between the Ukrainian ocular preventative disease programs at the Ministry of Health and the program for the present study. The cohort study will initially include the >3000 liquidators whose real time dosimetry has already been confirmed by retrospective dosimetry and are part of the National Registry of the Ministry of Health. The number will increase as the dosimetry is verified. The examinations will be conducted at seven centers (Kiev, Kharkiv, Dnipropetrovsk, Donetsk, Zaporizhya, Kirovohrad, Poltava) equipped for slit-lamp evaluations throughout Ukraine (in dedicated clinics for liquidators). The results will be provided to the participants in the study. Representatives from each of the sites will receive training and instruction regarding standardized approaches. These are outlined in **"Standardized Facilities, Methods and Techniques Required to Assess Early Lens Changes and Cataracts in Human Lenses"**(page 11). The data will be coded so that upon transmission to the American arm of the study the identity of the individuals will not be known. The data will be jointly analyzed and statistically evaluated.

Aim 2: Beginning with selected individuals from the approximately 120 liquidators with acute radiation syndrome whose measured doses exceeded 1 Sv (see page 13) and the almost 1200 patients whose doses have been already defined (see page 9), **a comprehensive, long term, non-subjective** follow-up of the lens transparency status will be undertaken. The study will be conducted at the Liquidator Clinic (Ukrainian=*Polyclinic)* which is being equipped with the Zeiss Scheimpflug Slit Lamp Imaging System and Oxford Retroillumination camera. Copies of the images will be provided to the American counterparts for corroborative analyses and cross indexing. The lenses of the patients will be documented at 6-12 month intervals. As potential patients become available from the cohort study, they will be added to the Scheimpflug assessment pool.

Aim 3: As cataract extractions become necessary for those whose history includes the Chernobyl experience, every effort will be made to recover lens epithelial tissue (tags) to be archived and eventually analyzed. The tissues will be recovered irrespective of whether or not the cataracts from which they were derived were radiogenic in origin. The effort will be integrated into the medium follow-up and can program to the Ministry of Health. The American counterpart will be responsible for processing and repositing the analyzed material. All results from the tag analysis will be shared among the participants.

The interactions of the various components of the program are shown in the flow chart above. The remote sites refer to institutes at the aforementioned cities which will be used to evaluate the individuals of the cohort study. The Liquidator Clinic will be the site for the non-subjective Scheimpflug Slit-Lamp Imaging and Retroillumination photography. These procedures will be overseen by Academician Sergienko of the Center for Eye Microsurgery (CEM) and indirectly by Academician Worgul and Dr. Medvedovsky of the Eye Radiation and Environmental Research Laboratory (ERERL). Professor Likhtaryov of the Ukrainian Scientific Center for Radiation Medicine (USCRM) will coordinate the dosimetric aspects and data correlations and Academician Kundiev, Director of the Institute of Occupational Health (IOH) will direct the diet, habit and occupational background survey and will provide for the overall administrative needs of the Ukrainian side of the program. Abbreviated biographies of all the primary participants are provided in the appedix.



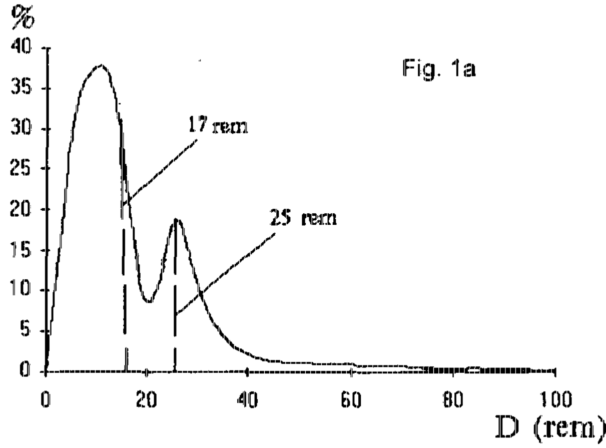
DOSIMETRY SUPPORT

Dosimetric Considerations: As illustrated in the organizational chart on Page 7, Prof. Ilia Likhtaryov Head of the Radiation Dosimetry Department of the Ukrainian Scientific Center for Radiation Medicine oversees the registry and retrospective dosimetry associated with the Liquidator follow-up. Although the total number of Liquidators exceeds 250,000 we will concentrate on the 130,000 who served during the period April 26, 1986 through February, 1987. The rationale for this selectivity rests on the fact that the dose distribution tends more towards the higher levels than is found to be the case for the Liquidators who came later. An explanation for the difference in exposure lies in the fact that until February, 1987 the workers were permitted to receive the accident dose limit of 25 cSv (see Figure 1a below) thereafter the permissible exposure was reduced to 10 and later 5 cSv.

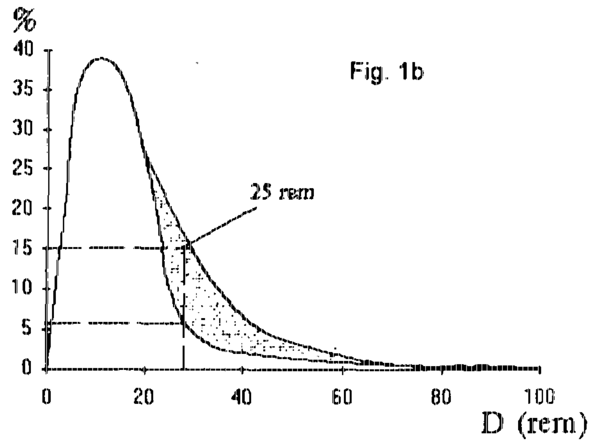
The following are the general criteria regarding the study population:

- Three groups of Liquidators who participated in clean-up inside the 30 km zone during 1986-1987 are being studied:
  - -civilians who worked inside the 30 km zone on a transient basis
  - -civilians who worked in the near the plant (<3 km from the Nuclear Power Plant)
  - -military workers (soldiers and officers), firemen, militiamen and others who worked in the immediate vicinity of the plant
- The main factors which determine the exposure level of persons in each of these groups are as follows:
  - -location of work relative to Reactor Number 4
  - -the nature of the work at the site
  - -time after the accident and time-interval of actual work

Of the **April, 1986 - February, 1987** Liquidators, approximately 130,000 live in the territory of the Ukraine. Of those about 22% have had direct measurements of dose. Unfortunately, the quality of these measurements varies greatly. An early effort to determine the dose-distribution resulted in the bimodal curve shown in Fig 1a on the right.



The second peak in this distribution, the so-called "administrative maximum", is the result of two limiting factors. One was the existence of a 25 rem "accident limit" in force until February 1986. Another was a strict policy of not recording the fact that the 25 rem limit was exceeded. By analyzing all the available data of direct measured doses the distribution curve (Fig. 1b) for the 1986-1987 population has been amended (from Likhtaryov, Personal Communication).



The highest probability of radiation cataracts is for those individuals exceeding doses of 25 cSv. Therefore, that group of individuals have been the initial focus of our cataract investigations (Fig. 1b). Estimating that 5-15% of the 130,000 1986-1987 Liquidators fall into this category, we project a potential pool of 6,000 - 20,000 people with exposures greater than 250 mSv. Given the fact that this population is already being so closely scrutinized and because their dose distribution is such that it maximizes the high dose group we plan to focus our effort on this group.

Table 1 on (below) provides a breakdown of age and dose for a group of 1197 Liquidators who have had confirmatory dosimetry as of May 1, 1993. This list, which is growing daily, illustrates the nature of the population which is available.

Almost all of the Liquidators have been examined in the Regional and Central (Kiev) Liquidator Clinics. An effort is on the way to include all the Liquidators in the special follow-up dosimetric investigation being conducted in the Dosimetry Division of the Central Clinic. The retrospective analytic dose revision, as well as the ESR study are essential features of the investigation. To date about 3000 of Liquidators with high doses (mainly the Power Plant workers) had been investigated using the detailed rout questionnaires (the analytic retrospective dosimetry). The results of the complete (ESR, etc.) dosimetric follow-up for 1197 Liquidators with high doses are represented in Table 1.

Table 1. Age and dose distribution for 1197 Liquidators (1% of the total population) who had worked near or inside Reactor 4 during May - June 1986 and whose doses were confirmed by follow-up retrospective dosimetry (Provided by I. Likhtaryov).

Number of Subjects							
Age (years)	Dose (cGy)						
	< 5	5 -10	10 - 25	25 - 50	50- 100	>100	All
18-22	2	1	8	8	2	4	25
23-30	1	4	22	42	56	22	147
31-40	4	10	44	116	146	70	390
40-55	8	22	80	184	208	112	614
>55	1	1	6	6	6	1	21
TOTALS	16	38	160	356	418	209	1197

It must be emphasized that these initial individuals were selected because of the high dose nature of their exposure. Therefore, this is not a random distribution. The skewing to the higher doses and older individuals is also not fortuitous. Those with supervisory responsibilities tended to be older and because of their roles were more likely to remain on-site for longer periods. This situation favors our study in that the population requiring cataract surgery will be drawn, for the most part, from the 40+ year old pool. In any case, one can readily appreciate the numbers which can be generated. It is hoped that by year's end 30,000 will have had complete retrospective corroborative dosimetry. For the purposes of the present study, as cataract patients are referred to CEM for extraction, potential candidates will be subjected to the dosimetric follow-up prior to surgery.

RATIONALE AND DESIGN

Specific Aim 1: **To obtain reliable evaluations of the risk of cataract development in a critical population involved in the Chernobyl accident.**

Review and Significance:

Various studies of radiation effects on the eye have concluded that characteristically the initial lesion caused by ionizing radiation is the posterior subcapsular (PSC) cataract (Miller et al., 1967; Merriam and Worgul, 1983). In terms of "spontaneous" cataracts attributable to other causes, PSCs are relatively infrequent, constituting only 15-20% of total cataracts (Sperduto and Hiller, 1984). The relatively low background rate of occurrence makes it easier to statistically detect an excess risk.

Radiation cataract was the first late effect of radiation to be documented among the Japanese A-bomb survivors (Cogan et al., 1949). The current epidemiologic database on radiogenic cataracts is relatively small and is marked by methodological limitations, some of which may have led to a questionable characterization of the risk function. For instance, the classic study by Merriam and Focht, based on a series of patients with prior radiotherapy, reported a threshold dose of 200 cGy (Merriam and Focht, 1957). However, only 33 patients with lens doses below 200 cGy were examined for cataracts in that study. The statistical power for detecting a modest sized risk with this small number of patients would be extremely low. The value of 200 cGy has nevertheless been commonly used by various radioprotection groups (ICRP, NCRP, UNSCEAR, BEIR) as an assumed threshold value for acute exposure, with even higher threshold values assumed for fractionated exposures.

In a recent reanalysis (Otake and Schull, 1990) of a 17-year collation of data on PSCs among 2,124 subjects in the Japanese A-bomb study, the authors applied both non-threshold models that were linear or linear-quadratic in dose and models that contained dose thresholds. They found a slightly better fit from a dose threshold model (with an estimated threshold of 70 cGy) than from the non-threshold models, but the difference between the goodness-of-fits was small and far from statistical significance, and, in fact, the non-threshold linear-quadratic model provided an adequate fit ($p = 0.18$) to the data. It is of interest that there was a suggestive elevation of risk in their lowest dose group of 1-99 cGy, such that the relative risk was 2.1 ($p = 0.08$, 95% confidence interval 0.8-5.8) according to our calculations. It is also noteworthy that this, the largest epidemiologic study of radiogenic cataracts, had significant limitations in that it was based on only 792 persons and 15 recorded PSCs in the dose range 1-99 cGy, and that the data were collected by various investigators which means that the ophthalmologic examinations were probably of variable sensitivity and quality (e.g., the most recent examinations were performed without pupil dilation (Choshi et al., 1983). The most recent report, which found a better fit for a non-threshold linear dose-response function than from a threshold function, is difficult to interpret because it lumped together significant and very minor lens changes (Otake et al., 1992).

At the other extreme from those who regard 200 cGy as the threshold dose, Klein et al (1993) recently related PSC prevalence to anamnestic reports of diagnostic radiation. They reported that PSC was related to a history of CAT scans (RR = 1.45, 95% confidence interval 1.08-1.95) and was suggestively related to diagnostic procedures to the head (RR = 1.27, 95% CI 0.96-1.66). However, these data should be interpreted cautiously, since the diagnostic radiation history was based on self-reports which are subject to recall bias.

Several studies have reported an inverse association between radiation dose and time to cataract presentation (Otake and Schull, 1990; Merriam and Focht, 1957; Merriam, Szechter, and Focht, 1972). This implies that there should be a long-term follow-up in a study that attempts to assess radiation risk of PSCs at low doses. This is one of the reasons that we are planning a 15-year study (i.e., with total follow-up for about

22 years after exposure), with the potential to extend the study to 25 years (total follow-up 32 years) if it seems warranted. Some (Choshi et al., 1983), but not all (Merriam and Focht, 1957; Otake and Schull, 1982), studies have also reported that the sensitivity of the lens to cataract induction is inversely associated with age at radiation exposure. This will be investigated as part of the proposed study.

A variety of other risk factors for cataracts have been reported. These include (see Bellows and Bellows; 1975) diabetes, diastolic hypertension and hypertensive drugs, phenothiazines and other major tranquilizers (Isaac et al., 1991), hypocalcemia, corticosteriods (Italian American Cataract Study Group, 1991; Jacques and Chylack, 1991), ultraviolet radiation exposure (Italian American Cataract Study Group, 1991; Leske and Chylack, 1991), cigarette smoking (Christen et al., 1992; Klein et al., 1993) and alcohol consumption (Munoz et al., 1993; Ritter et al., 1993). These factors need to be investigated as hypotheses in their own right - because some of the evidence is marginal or inconsistent - and also as potential confounder variables in analyzing radiation effects.

Intake of several nutrients may lower cataract risk: carotenoids, vitamin A (Hankinson et al., 1992) and vitamin C (Jacques and Chylack, 1991; Hankinson et al., 1992) - although not all studies have been positive for these nutrients (Italian American Cataract Study Group, 1991; Hiller et al., 1983). The dietary factors merit further investigation, and the present population may provide an especially good opportunity to do so because the range of vitamin intakes is likely to be greater than in studies in the U.S.

METHODOLOGY

This study will be the largest study ever conducted of radiation-induced lens opacities in a population with measured doses. It will have a range similar to the Japanese A-bomb ocular studies but will be 4-5 times as large (Choshi et al., 1983; Otake and Schull, 1990).

To achieve Aim 1, annual ophthalmologic examinations will be conducted among a cohort of 10,000 liquidators to document posterior subcapsular opacities and other opacities of the lens. As indicated in **Organizational Structure** (Page 6) this task will center on the application of conventional slit lamp biomicroscopy in seven centers which serve the medical needs of 90% of the surviving liquidators.

We will begin the study by examining first the >3000 liquidators (see **Epidemiologic Study Design and Protocol** - page 13) for whom real-time dose measurements had been confined by retrospective dosimetry and who are available for follow-up. Representatives from each of the centers will be trained at the CEM by Prof. Sergienko and Professor Worgul and the members of the staff of the Eye Radiation and Environmental Research Laboratory (ERERL). Professor Sergienko, Professor Worgul and Dr. Medvedovsky will monitor data acquisition by occasional on-site visits. In addition, the centers will be expected to refer cases to the Liquidator Clinic in Kiev for potential inclusion to the non-subjective quantitation analyses pool (***Specific Aim 2***). The referred cases will provide additional opportunities to verify compliance to the standardized criteria.

Central to the study are:

I. The standardization of the monitoring methodology
II. The size/dose distribution of the cohort group

I. Standardized Facilities, Methods, and Techniques Required to Assess Early Changes and Cataracts in Human Lenses

1. All ophthalmologists involved in this study must participate in special seminars organized by Ukrainian and American research team on procedures and the diagnoses of radiation cataract.

2. Slit lamps will be situated at the sites selected to conduct preliminary and clinical examinations (See page 6).
3. Standardized forms (see appendix) will be used by all the study examiners.
4. Slit lamp examinations will be performed with maximum pupil dilation. i.e., >5 mm..
5. All changes (dots, vacuoles, isolated or diffuse opacities, etc.) will be documented and indicated in the appropriate spaces on the standardized forms. See appendix.

Early lens changes

1. Polychromatic sheen associated with the posterior capsule.
2. Individual dots or vacuoles (less than 5) in c (a, p, e, s) or n. Number? [1-5].
3. Multiple (greater than 5 and fewer than 10) dots or vacuoles in c (a, p, e, s) or n.

Glossary: c: cortex, a: anterior subcapsular changes, p: posterior subcapsular changes, e: equatorial changes, s: supranuclear zone, n: nucleus.

Example: changes in the cortex and the posterior subcapsular zone will be scored as "cp"

Cataract stages:

While not pathognomonic Radiation cataracts develop in a characteristic sequential fashion. The sequela has served as the basis of the Merriam/Focht (1962) semi-quantitative scoring technique. A myriad of modified versions of the method have used successfully by a number of laboratories studying radiation cataract. The major strength being its unambiguous, albeit conservative, nature.

We will employ a modified Merriam/Focht scoring method to stage the cataracts using the same glossary as above for additional localization.

Stage 1+: Discrete opacity (a small spot seen with retroilluminated light; aggregate of dots, cortical spoke, watercleft; granulated opacities or vacuole aggregation in c (a, p, e, s) or n. ***Very Important*** - changes in the posterior subcapsular zone -could be the first sign of complicated (radiation) cataract. *Example: Stage 1+ cp.*

Stage 2+. More extensive cortical changes (collectively occupy the equivalent of 1/8 to 1/4 of the lens; if less - Stage 1.5). *Example: Stage 2+ c (a, p).*

Stage 3+. Advanced changes. Light does not reach vitreous (if some areas are semitransparent a 2.5+ grade is required).

Stage 4+. Premature cataract. Near-total lens opacification. In some areas it is possible to see the nucleus or posterior co of the lens.

Stage 5+. Mature cataract. Total lens opacification..

6. At the Kiev Liquidator Clinic, photo-documentation of lens changes in posterior subcapsular or cortical area should be instituted as described in the standard photo-record documentation sequence.
7. A specially selected subgroup of patients (Specific Aim 2) will be followed in the Liquidator clinic by Scheimpflug Slit Lamp System and retroillumination photography every six to twelve months by ophthalmologists with special training (at the Eye Microsurgery Center, Kiev and/or Columbia University, New York).

II. Epidemiologic Study Design and Protocol

The study design is basically a cohort study in which the subjects have a broad range of doses to the eye (from <5 to >800 cGy). Although the average dose to the entire Liquidator worker population is only 15 - 20 cGy, because the population is large we can oversample the medium and high dose ranges to produce a dose distribution that yields maximal statistical power to answer the scientific questions that we have posed. In order to obtain more detailed information on risk factors for cataracts, we will supplement the cohort study with a nested case-control study, in which a more extensive questionnaire will be administered to cataract cases and a set of matched controls.

Expected Risk of Cataract Induction: Based on both animal data (see Worgul et al., 1993) and preliminary indications in available human data (Otake et al., 1992; Klein et al., 1993) we posit that the radiation induction of cataracts is a stochastic process, which implies that even low to moderate doses will confer some measure of added risk. In order to estimate the likely magnitude of risk, we obtained preliminary data from Dr. Vasily Gaiday who has examined 120 "high dose "Liquidators in the Ukraine over the last five years (i.e., about 2-7 years post exposure) The examination techniques were not as sensitive as we will employ, so his numbers undoubtedly represent a lower bound on risk. The resulting data are shown in Table 2. The apparent absence of risk in the lowest dose group is likely to result from a combination of (1) the small number of persons examined, (2) the limited sensitivity of the examination procedure (see Table 2 footnote), (3) the fact that these examinations were performed within a few years after the radiation exposure and unfortunately he did not indicate when during the five years the individual observations were made. The last point is extremely relevant in that it is well documented that the time to cataract formation (latent period) is inversely related to dose.

Table 2. Preliminary Data for Cataractogenesis in 120 "High Dose" Liquidators
(Personal Communication, Vasily Gaiday)

Dose	n	Cataract	Non-cataract
1-2 Gy	59	0	59
2-4 Gy	45	8	37
> 4 Gy	16	6	10

It should be noted that the data are intrinsically anecdotal. Also, they are derived from observations spread out over the last five years. In addition, some of the cataracts were assessed grossly or with an ophthalmoscope. Therefore, the numbers are surely an underestimation of the individuals with early to moderate cataracts.

The average absolute risk in Table 2, calculated as a weighted average across the three dose groups, is about 5% per Gray (or 5 x 10^{-4} cGy^{-1}). This corresponds well with the absolute excess risk estimate of about 4.6% per Gray for the gamma dose found in the Japanese A-bomb study (Otake and Schull, 1990).

Dose Distribution and Selection of Study Subjects

Since >100,000 liquidators are available for study, the cohort will be selected so as to maximize our ability to detect the effects we are interested in. Specifically, we anticipate using approximately the dose distribution shown in Table 3 (Page 14).

Table 3. The distribution of doses that the study would aim for and that which was used in the simulation to evaluate statistical power.

Dose Range (cGy)	Assumed Mean Dose (cGy)	Assumed Percent Distribution	Distribution of Workers for N=10,000
< 5	2.5	20.0	2,000
5 - 10	7.5	5.0	500
10 - 25	17.5	20.0	2,000
25 - 50	37.5	30.0	3,000
50 - 100	73.0	17.5 *	1,750
> 100	130.0	7.5	750

* When just the dose range 0-70 cGy was considered, only half the subjects in the 50-100 cGy dose interval were used and the estimated mean dose assigned to them was 60 cGy.

This dose distribution of persons was chosen for the following reasons.

- 1) In order to have good statistical power and a good estimate of the risk at high doses, one needs a fair representation of high-dose (i.e., >70 cGy) persons. However, the number of high-dose persons is limited-about 2,600 with doses >70 cGy among the 130,000 liquidators-so our selection is limited by that number.

- 2) The main concern of the study is the risk among those with low to moderate doses, so we clearly want substantial numbers in the range of 10-70 cGy. (At doses below 10 cGy the number of subjects required to see an effect would probably be so large as to be prohibitive, especially if the dose-response function is linear-quadratic, so we have not chosen to follow many workers in the 5-10 cGy range.) In particular, we want to determine whether a dose-response trend can be seen over the range of 0-70 cGy, and to have reasonable power and precision in estimating the linearity/curvilinearity of the dose-response function.

- 3) An adequate baseline rate of cataracts among persons with equivalent intensity of screening is also needed as an anchor to the curve. To this end, a pool of liquidator subjects with little or no exposure will be included. Our projected sampling of workers by dose range represents an attempt to balance these three competing goals. The percents shown in Table 3 were applied to each of the sample sizes we evaluated: 5,000, 10,000 and 15,000.

Assessment of Statistical Power and the Required Sample Size

The preliminary study of the magnitude of radiation cataractogenesis among Chernobyl liquidators yielded an estimate of 0.0005 per person per cGy within seven years of the accident (see page 17). To be conservative we assume that the excess prevalence of 0.0005 per person per cGy would occur in 10 years i.e., that the excess is 0.005 per person-year per Gy [0.005 $(PY{\cdot}Gy)^{-1}$]. Given that our examination techniques will be more sensitive than those used with the preliminary data, we expect that the absolute excess risk will be higher. Nevertheless, to evaluate statistical power, we have used the estimate of 0.005 $(PY{\cdot}Gy)^{-1}$, plus values which bracket this estimate, namely, 0.01 $(PY{\cdot}Gy)^{-1}$ and 0.002 $(PY{\cdot}Gy)^{-1}$, in order to cover a plausible range of possible outcomes. Experimental studies by the present authors (see Worgul et al., 1989; 1993; Brenner et al., 1991; 1993) have shown that the dose-response curve is usually linear-quadratic, with the low-dose risk typically a factor of 1.2 to 1.8 lower per unit dose than the high-dose part of the curve. So, to be conservative in modeling the expected cataract response, we built in a low-dose reduction factor of 2 for doses under 70 cGy (where 70 cGy was the estimated "dose threshold" or inflection point in the A-bomb study dose-response curve (Otake and Schull, 1990.).

From the time of the Chernobyl accident until the end of our proposed 5-year study represents 12 years of follow-up (or 11.8 years on average once sample attrition is factored in).[1] A 15-year study, as we anticipate, would represent a maximum follow-up of 22 years and an average follow-up of about 19.6 years after factoring in sample attrition while a 25 year study would provide up to 32 years of cataract documentation. These values were applied to the risk estimates (e.g., 19.6 yr x 0.005 $(PY{\cdot}Gy)^{-1}$) in modeling the radiation risk. We applied the risk estimates to 5,000, 10,000 or 15,000 persons, using the dose distributions shown in Table 3. The assumptions and procedures for estimating the spontaneous prevalence and incidence of PSCs and for other aspects of the model are given below.

The statistical power calculations were performed using an adaptation of a method proposed by Nam (1987) to estimate the required sample size for a dose-response function with a binary disease outcome. When considering the full dose range, we modeled a function in which those above 70 cGy had the full coefficient of risk, while the risk for those below 70 cGy was 1/2 as large per unit dose. (For the dose interval 50-100 cGy (Table 3), we assumed that half the subjects were ≤ 70 and half were >70 cGy.) Since the range <70 cGy is of primary interest, we also estimated the statistical power for dose-response analyses limited to that dose range.

For the proposed 5-year study (i.e., for a total of 12 years since exposure), a sample of the expected results from the simulation of the spontaneous and radiation-induced PSCs is given in Table 4, and the statistical power results are given in Table 5. The statistical power was good for analyses over the full dose range. In particular, if the radiation risk of PSC cataracts is in the range 0.005 to 0.01 $(PY{\cdot}Gy)^{-1}$, then the statistical power was >99% for all three sample sizes considered, *viz*., 5,000, 10,000 and 15,000. For a radiation risk of 0.002 $(PY{\cdot}Gy)^{-1}$ the statistical power was >95% for the two larger sample sizes, but fell to 74% for 5,000 subjects.

1 Sample attrition represents losses to follow-up caused by death, severe disability, out-migration, terminated participation, etc.

Table 4. The numbers of expected spontaneous and radiation induced cataracts by dose range for selected sample sizes, study lengths, and risk coefficients.

	5 Year Study						10 Year Study					
	N = 5,000			N = 10,000			N = 5,000			N = 10,000		
	Spont-aneous	Radiation Induced		Spont-aneous	Radiation Induced		Spont-aneous	Radiation Induced		Spont-aneous	Radiation Induced	
Dose Range (cGy)		Risk Coeff# 0.0005	Risk Coeff# 0.03		Risk Coeff# 0.005	Risk Coeff# 0.03		Risk Coeff# 0.005	Risk Coeff# 0.03		Risk Coeff# 0.005	Risk Coeff# 0.03
< 5*	34.0	0.9	5.3	67	1.8	11.0	61	1.5	8.8	122	2.9	18
5-10*	8.4	0.6	3.3	17	1.1	6.6	15	0.9	5.5	30	1.8	11
10-25*	34.0	5.1	31.0	67	10.0	62.0	61	8.6	52.0	122	17.0	103
25-50*	50.0	17.0	99.0	101	33.0	198.0	91	28.0	166.0	182	55.0	331
50-100*	29.0	25.0	150.0	59	50.0	300.0	53	42.0	251.0	106	84.0	501
> 100*	13.0	29.0	172.0	25	57.0	344.0	23	48.0	287.0	46	96.0	574
Total	168	77	461	335	154	922	304	128	769	608	256	1,538

The risk coefficients are absolute excess risk per person-year per Gy.
* For those under a dose of 70 cGy the risk coefficient applied was only one-half the nominal value to simulate a low-dose reduction factor of 2.

Table 5. Estimates of the statistical power dose-response analyses for a cataract cohort study, for various sample sizes, study lengths, and risk coefficients.

Cataract	5 Year Study			15 Year Study			25 Year Study		
Risk Coeff.#	N = 5,000	N = 10,000	N = 15,000	N = 5,000	N = 10,000	N = 15,000	N = 5,000	N = 10,000	N = 15,000
FULL DOSE RANGE*									
0.002 #	74	95	99	89	99	99	92	99	99
0.005 #	99	99	99	99	99	99	99	99	99
0.01 #	99	99	99	99	99	99	99	99	99
0.03 #	99	99	99	99	99	99	99	99	99
DOSE ≤70 cGy*									
0.002 #	17	30	42	24	43	59	26	46	63
0.005 #	65	91	98	83	98	99	87	99	99
0.01 #	98	99	99	99	99	99	99	99	99
0.03 #	99	99	99	99	99	99	99	99	99

The risk coefficients are absolute excess risk per person - year per Gy.
* Because a low dose reduction factor of 2 was incorporated, the risk coefficients for doses ≤70 cGy were actually half as large as the nominal ones stated here.

The statistical power for the dose range ≤70 cGy in the proposed study was variable (Table 5). (Note that the risks actually being modeled in these analyses were only half the nominal values, due to the low-dose reduction factor of 2). For all the sample sizes considered and all the lengths of study, the statistical power was good if the radiation risk was at least 0.005 $(PY{\cdot}Gy)^{-1}$, except for the a 5-year study with a risk of 0.005 $(PY{\cdot}Gy)^{-1}$. However, if the radiation risk coefficient is only 0.002 $(PY{\cdot}Gy)^{-1}$ (i.e., the projected risk with a low-dose reduction factor is only 0.001 $(PY{\cdot}Gy)^{-1}$), then the statistical power over the dose range ≤70 cGy would not be adequate. It is noteworthy, however, that if the actual risk level is only slightly higher, namely, 0.002 $(PY \cdot Gy)^{-1}$ rather than 0.001 $(PY \cdot Gy)^{-1}$, then the statistical power with 10,000 subjects becomes more acceptable - 60% for a 5-year study or 79% for a 15-year study. By way of contrast, a 5,000 subject study does not reach acceptable levels of statistical power (35% and 50% for a 5-year or 15-year study respectively). This leads us to conclude that a sample size of 5,000 subjects is inadequate, and that the study should include at least 10,000 liquidators, so that, if the radiation risk at lower dose levels should prove to be as low as an excess of 0.002 per Gy per year, we still would have a reasonable prospect of detecting the risk.

Epidemiologic Questionnaire: As part of the overall Ukrainian effort, a complete personal history, including activities during and after their Chernobyl exposure as well as health and occupational information, is available on all subjects involved in the Chernobyl follow-up. In addition a risk factor questionnaire will be administered to the study subjects to obtain information on various types of exposures they may have had in the past. This questionnaire will be modeled after one used by the National Cancer Institute [Health Habits and History Questionnaire; Diet History and Other Risk Factors] which is currently being adapted to the culture (e.g., common foods) of Ukraine. An occupational history will be taken, as well as a list of types of exposures or industries in which certain genotoxic exposures are likely. The list will include exposures to organic solvents (e.g., benzene, trichloroethylene), tar or combustion derivatives (exposure to polycyclic aromatic hydrocarbons), ethylene oxide, etc. Information will be obtained on the amount of ultraviolet exposure (Hiller et al., 1983; Italian American Cataract Study Group, 1991), including both occupational and recreational. Smoking (Christen et al., 1992; Klein et al., 1992) and alcohol consumption (Ritter et al., 1993) will also be assessed. A dietary history instrument will be included, oriented toward assessing dietary (and diet supplements) of antioxidant micronutrients including Vitamin E, Vitamin C and carotenoids, as several studies have found these to be protective against cataracts, and they might be protective against micronucleation as well (Italian American Cataract Study Group, 1991; Jacques and Chylack, 1991; Hankinson et al., 1992; Leske et al., 1992).

The questionnaire on lifestyle factors (smoking, alcohol, ultraviolet exposure), medical exposures and dietary factors will be pilot tested and revised as needed. Questionnaires used in other diet studies in the F.S.U. (e.g., by Dr. D.G. Zaridze, Russia) will be solicited as models, to help ensure that the principal foods are captured in the questionnaire. However for the most part a culture-corrected version of the *Health Habits and History Questionnaire; Diet History and Other Risk Factors* developed by the National Cancer Institute will be the backbone of the survey.

Because obtaining a thorough set on all these risk factors would take a great deal of interviewing time it is not feasible to do for all 10,000 study subjects. We, therefore, propose that we will administer an abbreviated questionnaire (e.g., only about 50 dietary items and a limited set of questions on U.V. and occupational exposures) to all subjects. We will then administer a more extensive questionnaire to subjects with Stage 1+ cataracts or greater and to a set of matched controls. Two time-matched controls will be selected per case matched on age and sex (Lubin and Gail, 1984; Robins et al., 1986). The dosimetric estimates will also be carefully examined for these subjects and refined as much as possible. The interviewers and dosimetrists doing the case control work will be blinded as to case control status, so as to prevent an inadvertent biasing of the data.

Quality Control and Study Management

The cohort study subjects will be group matched on age across the dose range, so as to maximize the validity of the dose-response comparisons. Because diabetes is such a potent risk factor for cataracts, diabetics will not be enrolled in the study. Those who develop diabetes during the course of the study will be carried along in the study, but will be flagged and may have to be analyzed as a separate stratum or deleted from selected analyses.

Every effort will be extended to keep the participation rate high in the study. Since the liquidator clinics at which the examinations will be performed provide certain medical benefits, there should be incentive for them to continue their participation. We will carefully monitor the participation rates to prevent their varying by dose; if they do begin to do so, extra measures will be instituted to help equalize the rates.

A comprehensive plan for the management of the logistics and the data will be developed at the study outset. A computerized database will be developed to keep track of the addresses and most recent clinic visits of study subjects, so that reminders and other follow-up procedures can be instituted as needed. The results of the visual examinations will also be computerized. These databases will be maintained at the Ukraine Center for Radiation Medicine under the guidance of Prof. Likhtaryov. Quality control procedures for data collection, coding and computerization will be put in place at the study inception.

As mentioned above, quality control procedures for the ophthalmological examinations, and especially for defining opacities, is regarded as of utmost importance. To this end, initial training and periodic re-examination of a sample of subjects will be undertaken by Dr. Medvedovsky or Dr. Sergienko. As much as possible, examiners will be kept blinded as to the dose received by individual workers. A photo slit lamp will be used to obtain photo-documentation of cases diagnosed as having posterior subcapsular opacities, as well as a representative sample of 10% of the subjects. This will permit re-scoring of the opacities in a standardized fashion (e.g., as performed by Klein et al [1992]) and will also enable us to assess false-positive and false-negative screening rates.

Statistical Methods

Preliminary analyses will be conducted to determine whether any of the lifestyle-medical-dietary variables are confounders of the radiation-cataract association. Any that are found to alter the association by at least 10% will be included as confounders in the analyses. The main analyses will evaluate the dose-response relation between radiation and cataract occurrence, controlling for age and any confounder variables. The analyses will use a Poisson regression approach similar to the one used to study cancer mortality/incidence in the Japanese A-bomb study (e.g. Shimizu et al., 1990; Thompson et al., 1993). However, since this study will obtain both prevalence and incidence data, the methods will be modified to accommodate this feature, as was done in the recent Utah screening study pertaining to radiation fallout and thyroid neoplasms (Stevens et al., 1992). Linear, rather than log linear, models will be used for modeling radiation effects, since the exponential relationship implied by a log linear model is not biologically plausible.

ASSUMPTIONS AND METHODS FOR THE ANALYSIS OF STATISTICAL POWER

In order to have a realistic model of the expected frequency of "spontaneous" cataracts, several parameters need to be estimated: the prevalence and incidence of spontaneous cataracts at various ages,, the age distribution of the liquidators at the time of exposure, the losses-to-follow-up over time, and the resulting person-years-at-risk staged over time according to age. Analyses will also be conducted according to age at exposure, time since exposure (or attained age at risk), degree of dose protraction[2], and severity of the lenticular opacity.

Age distribution: The age distribution of the liquidators at the time of the Chernobyl accident is shown for a sample of highly exposed workers in text Table 2. Because we suspect that the ages shown there are

[2] Some of the workers received most of their dose in a single acute (<60 seconds) while others received theirs over weeks or months.

somewhat older than those for the liquidator population as a whole, a slightly younger age distribution was used in the calculations. For the age categories in text Table 2, the percents we assumed were: age 18-22 5%, 23-30 15%, 31-40 35%, 40-55 43%, over 55 2%.

Frequency of Spontaneous Cataracts: Since ionizing radiation exposure is associated primarily with posterior subcapsular cataracts (PSCs), we sought information on the prevalence or incidence of PSCs in relation to age and sex. Only three studies could be found that provide reasonably detailed data on PSC prevalence, and none on PSC incidence. These were the Framingham study (Krueger et al., 1980; Leibowitz et al., 1980; Sperduto et al., 1984), the Maryland Watermen Study (Adamsons et al., 1991) and the Beaver Dam Study (Klein et al., 1993). The differences in PSC prevalence according to sex were small and somewhat inconsistent across studies, so we used the combined-sex data in order to have more stable estimates. A summary of the age-specific prevalence proportions from these studies is shown in Table 6. The criteria for PSCs were apparently different in the Watermen study (Adamsons et al., 1991) from those in the other two studies, since the proportions were lower in the Watermen study. Since the number of persons evaluated was small, we relied mainly on the rates from the other two studies. The PSC prevalence proportions used for age-at-examination were: age ≤23 0.5%, 23-30 1%, 31-40 1.6%, 40-55 3.5%, 55-64 8%, 65-74 17%.

No data were found on PSC incidence.[3] However, methods exist to estimate age-specific incidence when one has the prevalence of a disease at various ages. An approximate method is given in reference (Podgor et al., 1983), namely,

$$I_j = (P_{j+1} - P_j) / ((1 - P_j) \cdot Y)$$

where I_j is the yearly incidence rate in the j-th age interval, P_j and P_{j+1} are the prevalence proportions in the j-th and j+1-th age intervals, and Y is the number of years in the j-th interval.

Further technical details and assumptions are given in Podgor et al. (1983). Using this formula we estimated yearly incidence rates of PSC occurrence for the various ages: ages 31-40 0.06%, 40-55 0.17%, 55-64 0.68%, 65-74 1.0%. An arbitrary incidence rate of 0.05% for ages ≤30 was used, because no data were available for making an estimate.

Table 6: Prevalence of Posterior Subcapsular Cataracts (PSCs) by Age

Age	PSCs/No. Subjects	%	Study
30-38	2/204	1.0	Watermen
40-49	0/145	0	Watermen
43-54	~24/1520	1.6	Beaver Dam
50-59	3/166	1.8	Watermen
52-64	50/1214	4.1	Framingham
60-69	6/177	3.4	Watermen
65-74	75/715	10.5	Framingham
70-79	3/105	2.9	Watermen
75-85	61/310	19.7	Framingham
75-84	~115/807	14.3	Beaver Dam
80 +	0/36	0	Watermen

3 Prevalence represents the proportions of persons with a disease at a given time, as might be found by a screening examination. Incidence, on the other hand, is the rate of occurrence of <u>new</u> cases of the disease over time, as might be determined, for instance, by a series of repeated screenings. The incidence rate is virtually always lower than the prevalence.

To simplify calculations, we assumed that the persons in each age-at-exposure interval, as shown in Table 1, were at the midpoint age of the interval and that our initial screening will occur 8 years after the exposure. We assumed the examination in year one was a prevalence screening, so that the age-specific prevalence rates applied to it, while all subsequent yearly examinations were incidence screenings so that age-specific incidence rates apply to these.

We assumed that attrition due to death, migration, termination of participation, etc. would be 30% by the end of a 15-year study. (Note, migration rates are quite low in the Ukraine, other than for the relocation that occurred due to Chernobyl, so this will be much less of a problem than it is in studies in the U.S.) We modeled the attrition by assuming that 70% would still be under follow-up at the end of 15 years, so we could calculate the average percent still under study for any arbitrary range of years. Under this assumption there would be an 89% follow-up rate after 5 years and a 55% follow-up after 25 years.

For the radiation-induced PSCs, it is not clear whether a relative risk model or an absolute risk model is more appropriate. We used an excess absolute risk model since absolute risk coefficients were derivable from the preliminary Chernobyl liquidator data whereas relative risks were not. We assumed a constant excess rate per year. In effect, we assumed that the initial screening examination, occurring 8 years after the radiation exposure, would detect the cataracts that had developed during the first 7 years, while subsequent screenings would each detect an extra year's worth of radiation-induced cataracts, out to 12 years for our initial 5-year study, to 22 years for a 15-year study, or to 32 years for a 25-year study. Modeling was performed using several different absolute risk coefficients - 0.0002, 0.0005, 0.001 per 10 years - in order to cover the range of plausible risks. The rationale for the coefficients is given in the text of the proposal.

Specific Aim 2: **To non-subjectively measure and quantify human radiation cataract development as a function of dose and establish a reference database which can be used to retrospectively assess radiation exposure in individuals.**

The Chernobyl accident provides an unparalleled opportunity to generate an image library of human radiation cataract development which is amenable to non-subjective quantitative analyses and longitudinal intra- and interpatient comparative analysis. In addition to the historically subjective nature of cataract scoring, the well known inverse relationship between cataractogenic expression and dose has been a major impediment to the establishment of risk. In fact, it is likely that the entire concept of cataract, as being a deterministic effect, may be derived from the limitations imposed by the latent period. A longitudinal long-term study of a dosimetrically well defined population, using state-of-the-art Scheimpflug Slit Lamp Imaging, should provide the data necessary to permit extrapolation of risk as a function of dose across age groups and remaining time at risk (life span). In addition, because the instrumentation provides digitally based images stored on long-lived optical media, the application of the technique promises the establishment of an image library which can serve a reference for future reanalysis and comparison. The use of the method also serves as the ideal validator for subjective follow-up studies, such as those planned in ***Specific Aim 1***.

The longitudinal follow-up will focus on the 1197 Liquidators whose doses have been well defined (see Table I in **DOSIMETRY SUPPORT** - page 8). The various statistical treatments have already been defined in the Discussion related to Specific Aim 1. In this case the nature of the data acquisition places an upper limit on the number of patients which can be imaged in a given period. A skilled ophthalmologist/technician may be able to image the eyes of ten individuals per day. Allowing for the inevitable problems associated with extensive examinations a lower number is more reasonable. It is anticipated that approximately 1000 individuals will be monitored using the technique. Most will be derived from the higher dose groups and representatives from each of the age subgroups listed in Table 1 (page 9) will be included

In addition to its non-subjective nature the methodology provides for a continuously incremental quantitative analysis of the loss of transparency over time. By generating a large number of cataract vs. time curves against dose the aim is to establish a series of standardized data set that can be used so that at a given time following the exposure the cataract severity can be measured and the dose estimated therefrom. This capability does not currently exist using the subjective non-linear scoring methodologies typically involved.

The equipment - a modified Zeiss Scheimpflug Imaging System and an Oxford Retroillumination Camera necessary for the quantitation - will be set up and operated in the Ophthalmology section of the Liquidator Clinic. The individuals selected for long term follow-up will be a subset of the cohort/case control study and will be derived from that population based on the confidence in the doses received, age, and cataract status. Cases which arise in the remote centers but of interest and relevance to this subproject will be referred to the Kiev clinic for evaluation and possible inclusion.

The study will involve several technologies which have the capability of producing a raw image database as well as non-subjective quantitative assessment of the images themselves. The use of the instrumentation will also provide the basis for technological and methodological improvements in human cataract assessment.

Specific Aim 3: **Establish a repository of tissue for immediate analyses and future reference. The effort will focus on calibrating the micronucleus assay in the lens as a potential retrospective dosimetric bioindicator for evaluating populations at risk.**

There exists a critical need to fund a program to acquire, reposit and analyze lens material retrieved during routine cataract operations from individuals whose history includes exposure to radiation from the Chernobyl incident. When a cataract is removed during a modern extracapsular cataract extraction (ECCE) procedure a small piece of lens epithelium, which we have come to call a "tag", is removed. This tag, typically discarded, contains a monolayer of 20,000 to 90,000 undifferentiated cell which, by virtue of their anatomical location, have remained so for most, if not all, of the post-natal life of the person from whom it comes. Thus, in a very real way, a tag is a *tabula vitae* which, irrespective of the basis for the opacity which occasioned its removal, can provide invaluable data on earlier damage caused by exogenous agents.

A member of the UACOS, the Eye Radiation and Environmental Research Laboratory (ERERL) has developed the means, and was the first to assess, human tags for cytogenetic damage using the micronuclei (MN) assay. Unlike the transient nature of induced MNs characteristic of more commonly used human tissues, e.g. peripheral lymphocytes, the unique biology of the lens guarantees that MNs which are produced by clastogens, such as ionizing radiation, are potentially retained for decades if not lifetimes (see Worgul et al., 1991). Our experiment studies (Odrich et al., 1988; Worgul et al., 1989; Tao et al., 1993) show that, as has been demonstrated for the blood and marrow, lenticular MNs are useful bioindicators of exposure to radiation. In the case of humans the tag MN assay has the added advantage of extreme longevity. It should be emphasized that the MN assay is only one of the quantitative parameters which can be derived from the tags. Cell density, cell size, and DNA content can and should be catalogued. Furthermore, our laboratory is perfecting the methodology for studying tags using DNA Fluorescence *In Situ* Hybridization (FISH). Thus, the tags can serve as a resource for studying fundamental aspects of radiation injury to human tissues and cells *in vivo*.

In any case it is absolutely essential that we stop the wholesale loss of this precious resource clearly being removed from the Chernobyl population. Our eventual goal is to use the MN tags first to calibrate the MN assay where the doses are well defined, and later to estimate exposure where they are not. However, an urgent priority must be to ensure that every effort is made to recover each and every tag as it becomes available, and provide for their proper handling. The following should be our immediate concern.

- **Organize and educate the appropriate Ukrainian sites for tag recovery, fixation, and record keeping.**
- **Establish a fixed specimen repository fully catalogued and cross-indexed with exposure registries such as that of I. Likhtaryov.**
- **As soon as possible remove the tissues from wet storage and prepare as flat-mounts to be archived in a permanent specimen library.**
- **Process select tags for quantitative analyses of MNs - dose calibrate the MN response in humans.**
- **Analyze and enter results in the Chernobyl database.**
- **Develop and implement a system whereby tags not utilized in the bioindicator follow-ups are available on a competitive basis, for basic science and applied studies. An example might be the application of specific methodologies of such FISH techniques to consider questions related to radiation damage in humans.**
- **Prepare protocols for the application of the technique to other populations at risk to radiation exposure, e.g., Chelyabinsk, Hanford and Savannah River, Wismut etc.**

The effort promises the following results:

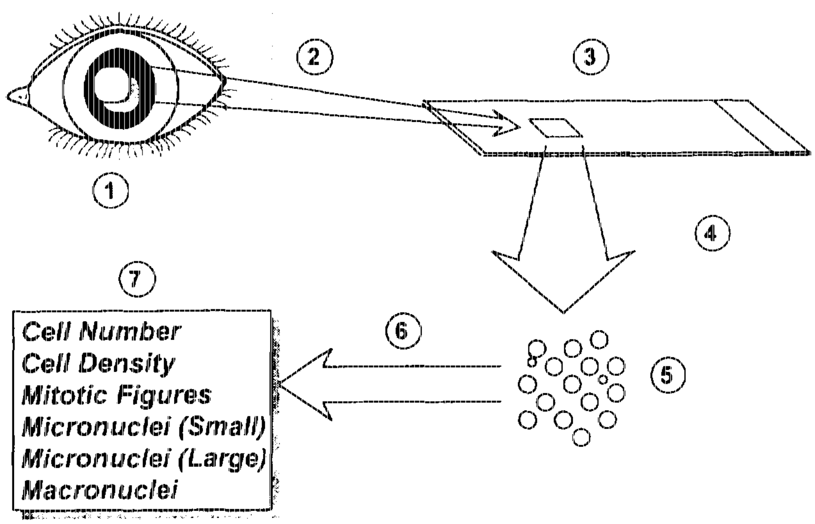
1. To preserve a component of the biological legacy of the Chernobyl accident for present study and potential future retrospective analyses.
2. To calibrate a bioindicator of human exposure to ionizing radiation at the individual and population level and use it to confirm estimated doses for individuals whose exposure is of low certainty. While only a very small percentage of the individuals whose tags are to be processed will have radiation cataracts, the opportunity to cross-calibrate two indicators (and perhaps others) of radiation damage will be extremely useful.
3. To use the data gleaned from the Chernobyl study to allow application of the technique to other populations at risk.
4. To further the appreciation of the biological consequences of acute and chronic radiation exposure.
5. To better understand the mechanism of action of radiation in a human tissue.

The general protocol for establishing the tissue repository is straightforward and is primarily an administrative task. The application of the MN assay, however, requires some explanation.

Tissue Source: As described in Organizational Structure and Dosimetry Support (pages 6 - 9) the infrastructure and organization is already in place for identifying candidates for study and tissue acquisition. The tags will be retrieved during extracapsular cataract extraction (ECCE) procedures conducted primarily at the Center for Eye Microsurgery (CEM) in Kiev, Ukraine. While plans are being jointly formulated to establish a repository for epithelia removed from all the Chernobyl exposed individuals, for the purposes of this proposal our aim is to select tags from those for whom the dosimetry is best defined including individuals who worked in the Chernobyl area but whose integrated exposure was not significantly higher than background. Another set of controls will be made up of people who had no Chernobyl experience. The latter group will generally be derived from populations residing in the Southeastern Ukraine. In selecting the exposed individuals, for the most part, our criteria will not be based on a primary diagnosis of radiation cataract, but more on the level of confidence regarding the dose which was received. For the purposes of the MN/dose response study it is not essential, or even necessarily desirable, that all of the cataracts which are extracted and included in the study are a direct result of radiation exposure. We are simply exploiting the opportunity to analyze lens epithelial tissue from cataracts which, for whatever reason, developed, but the history of which involves radiation exposure. Nonetheless, the nature of the opacity and other relevant information will permit the determination whether or not there is a "cataract dependent" association with MN profiles. Because bilateral ECCE's generally are not done at the same time we will make every effort to obtain the **second** (contralateral eye) tag from a given individual if and when it becomes available. This will allow to determine the "stability" of the MNs in the lens by virtue of the time disparity between the assessments. Since both eyes will have been exposed at the same time and both share very similar cellular kinetics (von Sallmann, 1952) the relative MN frequencies should provide data on the overall stability of the numbers which are present.

For the purpose of this study the material is coded so that the identity of the patient is known only to the primary physicians in Ukraine. The American component is supplied with critical information including personal history and habits, occupational background, and a nutritional/behavioral review based on a modified *Health Habits and History Questionnaire (HHHQ): Diet History and Other Risk Factors* from the NCI (1993 update). The questionnaire, still undergoing revision by Acad. Yuri Kundiev's group, will be used to attempt to account for possible confounding variables.

Following extraction the tags are preserved in Carnoy's fixative (1:3, glacial acetic acid:ETOH) for at least 1 but no more than 24 hours, after which the material will be maintained in 70% ethanol at 4°C until shipped to the United States. As soon as possible, following their arrival in the United States, flat mount preparations (see *Specimen Preparation and Analysis* below) will be made and the slides identified by the number code issued in Ukraine. At that point the specimen will be processed immediately or stored in a desiccator. The prepared flat mounts can be archived indefinitely in a desiccated state.



Specimen Preparation and Analysis: Over the years the ERERL has been involved in lens epithelial whole mount preparation studies and fine-tuning the technique for quantitative work. The availability of epithelial fragments or "tags" has opened new opportunities for studies in humans (Worgul et al., 1991). The general protocol is illustrated in the flow chart below.

1. *Tissue Retrieval*: The typical ECCE requires that, in order to gain access to the lens substance for removal, but at the same time preserving the zonule support of the capsular sac, a polygonal/circular window is made in the anterior capsule. The flap of epithelium/ capsule or tag is then placed on a surgical sponge and both are fixed together. While several capsulotomy techniques have evolved, to date we have found that the amenability of the tissue to flat mounting and later analysis is independent of the removal techniques and that all the methods produce equally usable tags. The tags, circular or polygonal (depending on the surgical technique used to remove them) typically have surface areas of up to 25 mm^2.

2. *Fixation*: Once removed, the sponge, with its adherent tag, is placed **immediately** (< 1 minute) in fixative (Carnoy's solution) for a minimum of 1-2 hours. The Carnoy's fixed material is transferred to a storage medium (70% ETOH) and maintained at 4°C if the tag is not to be mounted within 12 hours of extraction.

3. *Prepping*: The tag is mounted on a clean microscope slide **capsule side down**. This is done by unfurling the pliable tag in a drop of 35% ETOH on a microscope slide. Once positioned the excess ETOH is removed and the adherent tag allowed to air dry. If it is not to be processed immediately the mounted tag is stored in a vacuum desiccator. In our experience, tags stored desiccated for up to 2 years were unaffected. We believe that they can be maintained indefinitely in this manner. This is critical because we will be receiving material in a discontinuous fashion but always in large numbers (for convenience and cost effectiveness Prof. Sergienko's group will ship the material after accumulating several dozen vials). The tags will be prepared as soon as possible upon receipt. One person can comfortably prepare 12 flat mounts per day, a rate which far exceeds the ability to stain and analyze them. Therefore, the mounted tags will be archived in a desiccated state until processing.

4. *Staining*: The tissue will be stained for quantitative analyses. They will be stained by a modified Feulgen method (Worgul and Rothstein, 1974; Worgul et al, 1991) or with chromomycin A_3 (Jensen, 1977; Barrett et al., 1979; Worgul et al., 1991). Both methods, which stain DNA stoichiometrically, are used routinely in our laboratory. The actual staining procedures are detailed in the above citations.

5. *Imaging*: The stained preparations will then analyzed by using one of two systems.

A. We routinely assess Feulgen stained tissue using a Zeiss Photoscope III equipped with an ITC-3000® Image Analyzer (Image Technology Corp., N.Y.) and fully (x,y,z) automated (stage and focus) controller. Analysis is done in the visible spectrum (570 nm) with the wavelength controlled by a wedge monochromator.

B. Alternatively we utilize chromomycin A_3 which produces a nuclear-exclusive fluorescent signal when excited with 458 nm light. The advantage over Feulgen is that acid hydrolysis is not necessary and the cells are amenable to restaining with a second non-fluorescent dye. The fluorescent stained material is analyzable by one of two Zeiss Photoscope III setups employing epi- or trans-fluorescence excitation respectively. The epifluorescence unit is equipped with an MP-1 Zeiss photometry system. The transfluorescence set-up is in line with the ITC-3000® imaging system.

6. *Quantitation*: All the cells on each tag will be evaluated for nuclear anomalies indicative of an effect on the genome, i.e. small micronuclei (MN*sm*), large micronuclei (MN*lg*) and macronuclei (McN). MN*sm* are defined as round to oval, Feulgen or chromomycin A_3, positive structures with diameters ranging from 2 to 10% those of the parent nuclei. To minimize artifactual complication, it is important that the micronuclei be adjacent to, but distinct from, the parent nuclei, have discrete, smooth contours (suggesting a nuclear membrane) and that their staining intensities do not exceed that of the adjacent nuclei (Krepinsky and Heddle, 1983; Worgul et al., 1991). MN*lg* are micronuclei with the same staining criteria as above but with "sectional areas" of 10 to 30% that of interphase nuclei. McN are a small population of atypically large nuclei, almost invariably, of higher ploidy relative to the vast majority of the euploid (G_1-DNA content) cells in the epithelial populations. The integrated optical densities (IODs) of McNs exceed those of interphase nuclei by a factor of two or greater .

Several methods for actual counting have been employed in our laboratory. Each has its advantages and all three will be utilized to varying degrees in the context of this proposal.

A. *Manual scoring*: This is a highly labor intensive but extremely accurate means to obtain precise, absolute results. It requires that the scorer count all the nuclei on a specimen while recording the numbers of MNs (small and large), macronuclei and mitotic figures. Our experience has been that it can take from 16 to 80 man-hours to quantify one preparation depending on the size of the cell population (20,000 to 90,000 cells) present. Intra- and inter-scorer comparisons have shown that the absolute values tend to be reproducible to within ~3% and 5% respectively, with the relative ratios of anomalies to total cell numbers being about identical. This methodology has been employed in the study analyzing genotoxic damage in a randomized human cataract patient population (Worgul et al., 1991) and has been the basis of the study of the effects of exogenous physical and chemical mutagens on the lens epithelia of experimental animals (Odrich et al., 1988; Worgul et al., 1989; Tao et al., In Press). The major limitation of manual scoring resides in its labor intensive, low yield (few tags can be analyzed), nature.

B. *Semi-automated analyses*: More recently we have adopted a hybrid methodology which combines the bulk quantifying power of our imaging system with the discriminatory ability of a skilled observer. The strategy is to have a trained scorer first manually count the mitoses and aneuploids (micro and macro-nuclei) only. Following this, the total cell number on the tag is evaluated using the ITC 3000® image analysis system which, in a semi-automated manner, allows a relatively rapid counting of the 20,000 to 90,000 cells which comprise the tags. The total number of

G_1 nuclei plus macronuclei and the determination of the size of the tag permits the calculations for relative cell density and overall cell size. In addition, the relative number of macro and micronuclei can then be calculated on the basis of the total numbers of cells present on an individual tag basis. Since the tags have considerably fewer anomalies than the total number of nuclei (usually $<<1\%$), the scoring takes much less time. Using these means we can reduce the overall effort for each tag by an order of magnitude i.e. 2 to 8 man hours instead of the 16 to 80 hours required for manual counts. This has the advantage of making the total cell number less scorer dependent. It should be noted that the absolute value may be less accurate but it is exceedingly precise and highly reproducible. The diminished accuracy results from the reduced resolution due to the need for setting discriminating criteria to account for optical aberrations, artifacts, etc. However, the discriminators are themselves absolute and the G_1 nuclei are relatively homogenous in shape, size and coloration, so that the variation between individual determinations is very low, therefore, the increased precision. This has the overall effect of reducing errors in the ratios comparing anomalies against total cell numbers. Due to the fact that the nuclear anomalies are still assessed manually and because the cell number always represents a much larger component (often >100x), the greater consistency in each set of values results in an overall smaller difference in relative ratios. **The semi-automated methodology will be used routinely in evaluating the Chernobyl tags.**

C. *Fully Automated Analysis*: If the full potential of the application of MN analyses to human tags is to be realized it is important that we work towards greater efficiency in tag assessment. The possibility to process large numbers of tags will open the means to monitor population at risk on an almost routine basis. Furthermore, it will facilitate the use of animal surrogates. One can evaluate the tags of lens from feral animals captured in proximity to suspected sources of environmental contamination. Also, purpose-bred research animals can be used as sentinels in such locations. Such nature monitoring of human and animal populations necessarily require processing large numbers of tags which in turn recommends developing the means to analyze them in a fully automated system. Given the small size and relative heterogeneity of MNs automated assessment is a highly involved undertaking because the parameters which define the discrimination between the various nuclear morphologies against the artifactual "noise" become more critical.

The application of very sophisticated imaging systems to MN analyses of peripheral lymphocytes (the "classic" tissue used to assess MNs) is somewhat passed its infancy but has yet to achieve maturity. Nonetheless, the advances to date hold out great promise in their adaptability to the human lens epithelium because of the geometry of the system, i.e. a flat monolayer made up of a single cell type. Dr. William Blakely of the Armed Forces Radiation Research Institute (AFRRI) has developed a system which, with relatively little modification, has the potential to count MNs in human epithelia in a totally automated fashion.

The system uses digitized images of transilluminated phase contrast and specific DNA fluorescence acquired in several focal planes, which are then reconstructed to produce confocal-like images for the purpose of clearly delineating boundaries. Using software developed by his group, the microscope is under automated control and the data analyzed. Obtaining the yield of MNs/tag relative to the total number of cells with less than a 10% error is the initial goal. Tweaking the system to permit a high level of confidence for artifact exclusion and account for the McNs (distinguishing them from overlapping G_1 cells) is among the epithelium specific aspects with which he must deal.

Using the same material which are subject to our semi-automated quantification we will be able to determine the relative reliability of the Blakely system. Furthermore, both methods can then be cross-calibrated against fully manual quantitation. We hope that by the end of year two of this three year effort we will be able to recommend the use of a specific automated protocol for widespread applications.

7. *Data Analysis*: Using the **semi-automated analytic technique**, the MN frequency on a per epithelial tag basis will be determined. While clearly the emphasis of our effort will be on the well-documented MN indicator, the number of hyperploids (McNs) will also be scored. McNs, nuclei with DNA contents equivalent to tetraploids for the most part, but not infrequently octoploid and higher complements, occur in numbers which can exceed the MN population (Worgul et al., 1991). While clearly an effect on cytokinetics, the ultimate etiology of McNs, and their relevance to the genotoxicity and/or cataracts are completely obscure. Yet, their number, the fact that they reflect a cellular dysfunction, and their amenability to ready assessment, recommends that we include them in the analysis.

As is done classically, the numbers of MNs (*sm* and *lg*), as well as the McNs, will be scored, as will the total number of cells on each tag. The relative frequencies will be determined based on the total number of cells present. To address possibly subtler correlations we will also compare the ratios of MN*sm* vs. MN*lg* and McNs, an analysis which heretofore has not been done. We are confident that with the personnel and facilities dedicated to this project we will be able to process and quantify up to 200 epithelial tags each year. This will generate data from as many as 600 tags over the tenure of the grant. According to our calculations (see below) 600 tags are more than enough to arrive at a relative risk with good statistical power even using very conservative criteria.

Statistical Analysis: As discussed in the section entitled **Epidemiologic Questionnaire** (page 17) before performing the principal analyses of radiation effects, the possibility of exposures to other potentially confounding variables will be examined. In particular, data will be collected and analyzed on occupational exposures (e.g., ultraviolet, organic solvents, polycyclic aromatic hydrocarbons), lifestyle factors (recreational ultraviolet exposure, smoking, alcohol use) and dietary factors (Vitamins C and E and carotenoids). Any of these variables that affects the relationship between radiation and MN frequency by at least 10% will be included in the model. These variables will also be analyzed in their own right as risk factors or protective factors for MN formation and will be analyzed as possible effect modifiers of the association between radiation and MN frequency.

The data essentially consist of a count of number of MNs observed in each tag for each of several types of MNs (MN_{sm}, MN_{lg} and macronuclei [McNs]) plus the number of cells counted. This permits calculation, for each person, of the proportion of cells that have MNs of each type. For analysis purposes we will transform the proportions with the Freeman-Tukey variant of the arcsine transformation (Zar, 1984) in order to normalize their distribution. Standard linear regression can then be performed using the transformed data as the dependent variable and using estimated lens dose , plus dose squared, age and any potential confounding variables, as the covariates. It is then possible to back-transform the results to the original scale of proportions in order to estimate the approximate risk per unit dose on a natural scale.

The preliminary data suggest there may be extra-Poisson variability in the MN frequencies. Therefore, in order to eliminate the under influence of individuals with large numbers of MNs, we will also analyze the data with it scored dichotomously as positive or negative. The definition of positive will be chosen, based on the essentially unexposed subjects, as a proportion of MNs above the typical background level for the particular type of MN phenotype. These data will then be analyzed assuming a binomial distribution for each individual and modeling the binomial probabilities, as has been done by Tarone for chromosome aberration data (Kleinerman, 1990)

The analyses will focus on (1) the null hypothesis of whether there is a dose-related radiation effect, (2) the shape of the dose-response curve, and (3) the development of a calibration curve. The shape of the dose-response curve is of considerable interest since MN mutation is a biological indicator of genotoxic lens damage which may have a bearing on the radiation induction of cataracts. It is currently an open question, one of concern to the radiation protection community, as to whether the dose-response relation for cataract induction is a threshold or non-threshold phenomenon, and, if non-threshold, whether the dose-response curve is linear or quadratic (i.e., upward concave) for low-LET radiations. Information on the shape of the radiation dose-response curve for long-term mutagenic damage to the lens will help define the biological parameters for cataract induction. Perhaps more importantly, a goal of the study is to assess the usefulness of the MN assay as a biodosimeter. This involves assessing the standard errors on predicted doses given the bioassay results (i.e., a calibration curve approach [Draper and Smith, 1981]), and examining the sensitivity of the assay to relatively low doses (say, on the order of 10-50 cGy).

Sample Size Assessment: In order to perform a preliminary assessment of the needed sample size, we determined the ratio of average proportions of MNs which could be detected for different sample sizes. Ideally, one would want to examine this using a dose response type sample-size analysis. But, because statistical sample-size software was available for only the two group t-test, we used this as a surrogate, recognizing that the results of a two-group analysis will ***overestimate*** the size of the effect that would be required to produce statistical significance (Lagakos, 1988), so these results are conservative. We applied the Freeman-Tukey arcsine transformation to the proportions found in the baseline data collected by the present authors (Worgul et al., 1991 - appended) and performed sample size calculations for t-tests, assuming that half the subjects were "exposed" and half "unexposed" to radiation. We calculated the minimum detectable difference in arcsine-transformed means, using $\alpha = 0.05$, $\beta = 0.1$, one-tailed. We back-transformed the arcsine results to the original scale of proportions and calculated the ratio of the detectable mean proportion in the exposed group to the baseline proportion of the unexposed group, which we refer to as the minimum detectable ratio (MDR).

We found that , for the various categories of MNs (small, large, macro), the MDRs ranged from 1.3 to 1.4 for 600 study subjects. One can think of an MDR of 1.4 as roughly like being able to detect a relative risk of 1.4, which would represent excellent statistical power for an epidemiologic study. It should be emphasized that our planned dose-response analyses will be able to detect an even smaller effect than those shown by the crude dichotomized analyses. Although as shown in Table 7 as few as 400 tags may generate a good MDR we believe we could not make do with a smaller sample size than 600 cataract subjects. Detecting statistical significance is only the first step of the statistical analysis; our major interest is in obtaining as precise an estimate as possible of the dose-response relationship for the purpose of developing a calibration curve, which requires a large number of subjects (Ehrenberg, 1982). Nonetheless, the sample size is such that by about 2/3's into the project we would be able to increase the low dose sample size for greater resolution.

Table 7. The minimum detectable exposed/unexposed Ratios (MDRs) of the average proportion of cells with MN of several types in relation to total sample size (exposed plus unexposed, in equal numbers).

TOTAL SAMPLE SIZE	SMALL MN	LARGE MN	SMALL AND LARGE MN	McN
100	2.14	1.94	2.07	1.80
200	1.76	1.64	1.71	1.54
300	1.61	1.52	1.57	1.42
400	1.51	1.43	1.48	1.37
500	1.45	1.37	1.43	1.33
600	1.41	1.35	1.39	1.29

For the purpose of documenting possible confounders in the MN frequency assessment the following possible modulators will be considered in the analyses.

A. *TAC (Time After Chernobyl)*: As discussed in Preliminary Results, the time which has elapsed post-exposure is a potentially critical parameters because for MNs to appear an intervening mitosis must occur. The greater the interval, the greater the opportunity for a damaged cell to have traversed the cell cycle and produce an MN. But it should be appreciated that cells which contain MNs, in an extended time frame, may be eliminated from the population by death or during an attempt to differentiate. Therefore, cellular dynamics may be a source of error. Fortunately, in the case of the lens cell population this may not prove problematic. This is because we will be using tags which represent the central zone (CZ) of the epithelium (an area of great stability) due to low cell turnover and the lack of opportunity to differentiate. Also, our previous data (Worgul et al., 1991), have shown that if MNs are lost in that region, they must be removed at a rate substantially lower than they are created. Finally, in the planned studies most, if not all, of the tags to be used will have come from the liquidators who worked on the site in 1986-1987. These aspects should minimize the influence of time in the assessment. Nonetheless, every consideration will be given to its possible modulating role of the "Time Displacement Factor" (Savage, 1989).

B. *Total integrated dose*: The attractive feature of using the MN test for mutagenesis is its dose-dependence in peripheral blood lymphocytes for a spectrum of mutagens (see Heddle et al., 1983; 1991), including radiation (Ramalho et al., 1988; Gudi et al., 1990; Murray et al., 1991; Fucic et al., 1992). Our studies in experimental animals have not only shown a quantitative relationship with the clastogenic effects of radiation (Geard et al., 1987) but also the production of MNs (Odrich et al., 1988). It is very likely that MN frequency in the human lens will also correlate to dose. We need only to utilize the enormous resource which the Liquidators represent to demonstrate it. While the doses to be considered will span <5 mSv (equivalent to background radiation dose) to >1 Sv, every effort will be made to obtain a representative dose distribution with an emphasis, because this is the first study of its kind, on the higher dose group, during the first year of the study. Because currently 4/5's of Prof. Sergienko's CEM cataract patient population is made up of individuals not exposed at Chernobyl. It will be relatively easy for him to provide us with highly correlated non-exposed cohort (controls). An additional group of controls will include liquidators whose tasks did not result in a measurable increase over background in Kiev in the summer of 1986 ~450 mSv. The control inclusion will begin the second year of the study and at the termination, the total pool will be 60 tags (of the 600 tags total we plan to study). The rationale for delaying the acquisition of tags is to generate a population of exposed individuals and , armed with information about those, an effort will be made to match the control population in age distribution, health, and nutritional background. Since MN production is stochastic we do not anticipate a threshold. However, by paying particular attention to the exposure level, which exceed 0.3 Sv, we hope to maximize opportunity to generate a dose-response curve.

C. *ATE (Age at Time of Exposure)*: A potential modulator of the MN response is the age of the individual at the time of exposure. However, there are conflicting reports as to whether or not age has an effect on overall MN numbers for human lymphocytes (Fenech and Morley, 1985; 1986; Scarfi et al., 1990; Tomanin et al., 1991). Based on experimental animals one might think that growth kinetics will be a tremendous variable (see Rothstein, 1968; Harding et al., 1971). However, all indications suggest that human lens growth, after the second decade, levels off (Worgul, 1982; Worgul et al., 1989). Inasmuch as a substantial subset of any population which survives past the sixth decade will require cataract surgery, the epithelia from populations which are purported to have been, or are, suspected of being at risk of exposure to environment physical and clinical mutagens, can be assayed. Interestingly, Adamson et al., 1991, found that Posterior Subcapsular (PSC) Cataracts constitute a much higher percentage of the surgical population than the general population due, it is believed, to the more visually debilitating nature of such cataracts. But no matter what the basis of this propensity, the population is considered to be a likely expression of more recent damage because the fibers which constitute the most superficial region of the lenses are the latest to be deposited (Worgul et al., 1982; Worgul et al., 1989). Despite the low expectation of effect on the outcome of the analyses, age is a parameter which must be an integral part of our evaluation.

D. *Sex*: While there is little to suggest a great difference in radiosensitivity between the sexes in humans, for some agents and under certain conditions, MN induction has been shown to differ in male and female experimental animals (Collaborative Study Group for the Micronucleus Test, 1986; Holmstrom, 1990; Sutore and Sato, 1990; Tamura et al., 1990; Urlando and Heddle, 1990) and is suggested in at least one study on human lymphocytes (Tonanim et al., 1991). Therefore, the male and female data will not be pooled. For the purposes of this proposal this assurance is somewhat moot in that the vast majority of the liquidators (particularly in the high dose groups) is overwhelmingly male.

E. *Smoking*: For the purposes of this study we will request that the epithelia to be included in our study are derived from individuals whose health status is unremarkable. Although it would be preferred that only non-smokers are included, given the controversies surrounding the relative MN frequency in oral mucosa (Brenner et al., 1992) and blood (Tomanin et al., 1992; Nantos-Melly and Calvalcante, 1992) of smokers and the cloudy picture of smoking and cataracts (Christen et al., 1992; Hankinson et al., 1992), the national habit of cigarette smoking in the Former Soviet Union (FSU) countries like Ukraine makes it impractical to use, exclusively, non-smokers. The data will be analyzed with an eye towards smoking as a potential competing risk.

F. *Cataract:* See ***Specific Aims 1 and 2.***

FUNDING RESPONSIBILITIES AND REQUIREMENTS.

The joint effort involves three primary thrusts: a classic cohort cataract analysis with a nested case control study; an intra-case quantitative long-term integrative cataract follow-up; and a tissue repository/analysis section.

Ukrainian Contribution. Academician Yuri Spizhenko of the Ministry of Health of Ukraine has committed the resources necessary to support the Ukrainian side of the effort. This includes the establishment of the clinic for the ophthalmological follow-up of the population, the involvement of the six remote regional polyclinics integrated into the clinic in Kiev, the commitment of personnel to conduct the follow-up, and support for the institutes involved in the data acquisition and analysis in Ukraine. Because of a prohibitively skewed exchange rate, the acquisition of items which require foreign currency will fall mainly to the American side of the effort.

The United States Commitment. The United States contingent requires support for the staff of the Eye Radiation and Environmental Research Laboratory (ERERL) to oversee the program, to initiate a study on a United States control group, to analyze the data, to establish a database for ready reference, and to manage the tissue repository in all its facets. Dr. Roy Shore, who will advise us on epidemiological questions, will also require support. The Armed Forces Radiobiological Research Institute (AFRRI) under the auspices of the Defense Nuclear Agency (DNA) has provided the cooperative start up funds to obtain equipment for the conduct of ***Specific Aim 2***, the long-term quantitative follow-up of cataracts in the Chernobyl Liquidator population. This support is scheduled to continue through 1997, at which time continued funding will be required. Currently, there are no funds for the cohort, nested case control, study, nor are there funds to initiate the tissue repository and analysis.

Budget 1; *Specific Aim 1.* In order to undertake the major cohort study which we have outlined in this proposal a significant commitment on the part of the United States is required. The monies will be primarily for the salary support of Dr.'s Worgul, Medvedovsky, Shore, a fellow to conduct ophthalmological validation assays and one full time equivalent data specialist for data entry and database maintenance. The extensive and intensive nature of the study will require relatively frequent travel by some component of the American contingent for site visiting and progress assessment. Therefore, travel and communications will also represent a significant cost category. Because we will encourage photo documentation of cataracts from the population, photographic supplies, and developing costs are expected to be significant. We feel that at a minimum the Kiev Clinic (which will see about 50% of all the Liquidators) should be supplied with a modern Photo-slitlamp for cataract documentation and the possibility of independent analyses. For the purposes of reporting and record updating some secretarial assistance will also be required.

Budget 2, *Specific Aim 2.* as indicated above, the long term quantitative longitudinal follow-up of the liquidator population, utilizing non-subjective methodologies, has already been initiated and funding is anticipated through 1996. Depending on the quality of the data, the success of the follow-up and the status of the study at that time, additional funds will be sought to continue the effort.

Budget 3, *Specific Aim 3.* The effort is two pronged involving the establishment of a repository for the epithelial tags which are removed from individuals whose history includes exposure as a result of the Chernobyl experience. This effort is not limited to just the liquidator population, but all who have had any level of exposure. As regards the repository and database, there are two subsets to this effort. One is the simple acquisition, cataloging, and maintenance of the tissue in a fixed condition with an effort to begin to prepare the material as flat mounts for permanent storage. The personnel costs, which are the major expense, involve the administrative oversight, cataloging and data entry, and one technician level individual to begin flat mount preparations. Working on a full time basis, such an individual would be able to prepare 20 tags/day for permanent storage. This is consistent with the current rate of tag recovery. However, it is anticipated that in less than a decade the numbers will approach 100 per day in terms of acquisition and wet storage. It will be necessary to increase the effort to prepare the tissues for permanent archives. In addition to the personnel costs, low temperature freezers, explosion proof refrigerators, and large volume dissector chambers are required. An important aspect of the repository effort includes a retrieval section, which we will make available the sequestered material for research purposes. This represents an administrative corps plus an individual responsible for maintenance of the repository, retrieval and shipment of the material upon successful application. That individual will also be responsible for follow-up regarding the disposition of the material and the inclusion of the results of the studies in the overall Chernobyl database.

The second thrust of the ***Specific Aim 3*** is to use the tag material to conduct a specific study aimed at calibrating the MN frequency against dose in lens epithelia for the purpose of applying methodology as a bioindicator in other population with less defined dosimetric data. In additional to oversight and administration, the analysis will be heavily technician dependent inasmuch as current semi-automated, quantitative cytological/MN analysis of the tag requires a minimum of one person day per tag. Therefore, a skilled technician is able to process a maximum of approximately 200 tags per annum. We propose that initially two individuals be assigned to the task. During the course of the first year we will make an effort to train Ukrainian personnel to prepare flat mounts of tag. If this proves satisfactory there should not be an overall incremental cost despite an ever increased population of recovered tags as time goes on.

LITERATURE CITED

Adamson, I., Munoz, B., Enger, C. and Taylor, H. (1991). Prevalence of lens opacities in surgical and general populations. Arch. Ophthal. 109:993-997.

Al-Sabti, K., Lloyd, D.C., Edwards, A.A. and Stegnar, P. (1992) A survey of lymphocyte chromosomal damage in Slovenian workers exposed to occupational clastogens. Mut. Res. 280:215-223.

Anonymous (1982). Epidemiology of cataract (editorial). Lancet 1:1392-1393.

Ashby, J., Tinwell, H., Gulati, D. and Heddle, J.A. (1990). Overview of the study in relation to protocol design for the rodent bone-marrow micronucleus assay. Mut. Res. 234:223-248.

Barrett, D.L., Jensen, R.H., King, E.B., Dean, P.N. and Mayall, B.H. (1979). Flow cytometry of human gynecologic specimens using log chromomycin A_3 fluorescence and log 90° light scatter. J. Histochem. & Cytochem. 27:573-578.

Becker, P., Scherthan, H. and Zankl, H. (1990). Use of a centromere-specific DNA probe (p82H) in nonisotopic *in situ* hybridization for classification of micronuclei. Genes. Chrom. & Cancer, 2:59-62.

Bellows, J.G. and Bellows, R.J. (1975). Presenile and senile cataract - the aging lens: clinical considerations. In: Cataract and Abnormalities of the Lens. pp. 303-313, ed. J.G. Bellows, Grume and Stratton, New York.

Brenner, D.J., Medvedovsky, C., Huang, Y., Merriam, G.R., Jr., and Worgul, B.V. (1991) Accelerated heavy particles and the lens VI: RBE studies at Low Dose. Rad. Res. 128: 73-81.

Brenner, D.J., Medvedovsky, C., Huang, Y. and Worgul, B.V. (1993) VIII. Comparisons between the effects of iron ions (190 keV/μm) and argon ions (88 keV/μm). Rad. Res. 133:198-203.

Benner, S.E., Lippman, S.M., Wargovich, M.J., Velasco, M., Peters, E.J., Morice, R.C. and Honk, W.K. (1992). Micronuclei in bronchial biopsy specimens from heavy smokers: Characterization of an intermediate marker of lung carcinogenesis.

Choshi, K., Takaku, I., Mishima, H., Takase, T., Nerlishi, S., Finch, S. and Otake, M. (1983). Ophthalmologic changes related to radiation exposure and age in Adult Health Study sample, Hiroshima and Nagasaki. Radiat. Res. 96:560-579.

Christen, W.G., Munson, J.E., Seddon, J.M., et al. (1992) A prospective study of cigarette smoking and risk of cataract in men. JAMA 268:989-993.

Cinotti, A.A. and Patti, J.C. (1968). Lens abnormalities in an aging population of nonglaucomatous patients. Amer. J. of Ophthal. 65:25-32.

Cogan, D.G., Donaldson, D.D. and Reese, A.B. (1952). Clinical and pathologic characteristics of radiation cataract. Arch. Ophthal. 47:55-70.

Cogan, D.G., Martin, S. and Kimura, S. (1949). Atomic bomb cataracts. Science 110:654-655.

Collaborative Study Group for the Micronucleus Test. (1986) Sex difference in the micronucleus test. Mut. Res. 172:151-163.

Donaldson, D.D. (1976). In: The Crystalline Lens. C.V. Mosby, St. Louis.

Eiberg, H., Marner, E., Rosenberg, T. and Mohr, J. (1988). Marner's cataract (CAM) assigned to chromosome 16: linkage to haptoglobin. Clin. Gen. 34:272-275.

Eller, A.W., Day, R. and Gorin, M.B. (1993). A cross-sectional study of lens changes in children from exposed and non-exposed regions of surrounding the Chernobyl Nuclear Reactor. Inv. Ophthal. Vis. Sci. 34:1065.

Ehrenberg, A.S.C. (1982) Discussion of Brown, "Multivariate calibration". J.R.S.S. Series B. 44:311-312.

Fenech, M. and Morley, A.A. (1985). The effect of donor age on spontaneous and induced micronuclei. Mut. Res. 148:99-105

Fucic, A., Garaj-Vrhovac, V., Skara, M. and Dimitrovic, B. (1992). X-rays, microwaves and vinyl chloride monomer: their clastogenic and aneugenic activity, using the micronucleus assay on human lymphocytes. Mut. Res. 282:265-271.

Geard, C. (In Press). Cytogenetic assays for genotoxic agents. Lens and Ocular Tox.

Geard, C. and Worgul, B.V. (1987). The lens and cataract: Clastogenic responses in epithelial cells of the organ cultured rat lens. Environ. Mutag. 9:111-122.

Goldman, H. (1937). Studien uber den allerskernstreifen der linse. Arch. Augenheilk. 110:405-414.

Gudi, R., Sandhu, S.S., and Athwal, R.S. (1990). Kinetochore identification in micronuclei in mouse bone-marrow erythrocytes: An assay for the detection of aneuploidy-inducing agents. Mut. Res. 234:263-268.

Gudi, R., Xu, J. and Thilagar, A. (1992). Assessment of the *in vivo* aneuploidy/micronucleus assay in mouse bone marrow cells with 16 chemicals. Environ. and Molecular Mutagenesis, 20:106-116.

Hankinson, S., Stampfer, M., Seddon, J., Colditz, G., Rosner, B., Speizer, F. and Willett, W. (1992) Nutrient intake and cataract extraction in women: a prospective study. Br. Med. J. 305:335-339.

Hankinson, S.E., Willett, W.C., Colditz, G.A., et al. (1992) A prospective study of cigarette smoking and risk of cataract surgery in women. JAMA 268:994-998.

Harding, C.V., Reddan, J.R., Unakar, N.J. and Bagchi, M. (1971). The control of cell division in the ocular lens. Int. Rev. Cytol. 31:215-300.

Heddle, J.A. (1973). A rapid *in vivo* test for chromosomal damage. Mut. Res. 18:187.

Heddle, J.A. (1990). Micronuclei *in vivo*. In: Mutation and the Environment, Wiley-Less, Inc., London, pp. 185-194.

Heddle, J.A., Cimino, M.C., Hayashi, M., Romagna, F., Shelby, M.D., Tucker, J.D., Vanparys, P. and MacGregor, J.T. (1991). Micronuclei as an index of cytogenetic damage: past, present, and future. Environ. Mol. Mutag. 18:277-291.

Heddle, J.A., Hite, M., Kirkhart, B., Mavournin, K., MacGregor, J.T., Newell, G.W. and Salamone, M.F. (1983). The induction of micronuclei as a measure of genotoxicity: A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mut. Res. 123:61-118.

Heddle, J.A., Stuart, E. and Salamone, M.F. (1984). The bone marrow micronucleus test. In: Handbook of Mutagenecity Test Procedures, Ed. Kilbey,, B.J., Leganor, M., Nichols, W. and Ramel, C., Elsevier Sciences Publ., B.V., 2nd Ed., pp. 441-457.

Hiller, R., Sperduto, R. and Ederer, F. (1983) Epidemiologic associations with cataract in the 1971-1972 National Health and Nutrition Examination Survey. Am. J. Epidemiol. 118:239-249.

Hogstedt, B. and Karlsson, A. (1985). The size of micronuclei in human lymphocytes varies according to inducing agent used. Mut. Res. 156:229-232.

Holmstrom, M. (1990) Induction of micronuclei in bone marrow of mice exposed to 1, 2, or 3 daily doses of urethane. Mut. Res. 234:147-154.

Holsclaw, D.S., Merriam, G.R., Medvedovsky, C., Rothstein, H. and Worgul, B.V. (1989) Stationary radiation cataracts - as animal model. Exp. Eye Res. 48:395-398.

Holsclaw, D.S., Medvedovsky, C., Rothstein, H. and Worgul, B.V. (Submitted) Hormonal modification of clinical and cytopathological changes in radiation induced cataracts.

Hutchinson, F. (1973). The lesions produced by ultraviolet light in DNA containing 5-Bromouracil. Q. Rev. Biophys. 6:201-246.

Isaac, N., Walker, A., Jick, H. and Gorman, M. (1991). Exposure to phenothiazine drugs and risk of cataract. Arch. Ophthalmol. 109:256-260.

Italian-American Cataract Study Group (1991) Risk factors for age related cortical, nuclear, and posterior subcapsular cataracts. Am. J. Epidemiol. 133:541-553.

Jacques, P. and Chylack, L. (1991) Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. Am. J. Clin. Nutr. 53:352-355.

Jensen, R.H. (1977). Chromomycin A_3 as a fluorescent probe for flow cytometry of human gynecologic samples. J. Histochem. & Cytochem. 25:573-579.

Jose, J.G. (1978) The role of DNA damage, its repair and its misrepair in the etiology of cataract: a review. Ophthal. Res. 10:52-62.

Karim, A.K.A., Jacob, T.J.C. and Thompson, G.M. (1987). The human anterior lens capsule: cell density, morphology and mitotic index in normal cataractous lenses. Exp. Eye Res. 45:865-874.

Klein, B.E.K., Klein, R., Linton, K.L.P. and Franke, T. (1993) Diagnostic X-ray exposure and lens opacities: The Beaver Dam eye study. Am. J. Public Health 83:588-590.

Klein, B.E., Klein, R. and Linton, K. (1992). Prevalence of age related lens opacities in a population. The Beaver Dam Eye Study. Ophthalmol. 99:546-552.

Klein, B.E.K., Klein, R., Linton, K.L.P. and Franke, T. (1993) Cigarette smoking and lens opacities: the Beaver Dam eye study. Am. J. Prev. Med. 9:27-30.

Kleinerman, R.A., Littlefield, L., Tarone R., Sayer, A., Hildreth, N., Pottern, L., Machado, S. and Boice, J. (1990) Chromosome aberrations in relation to radiation dose following partial body exposures in three populations. Rad. Res. 123:93-101.

Krepinsky, A.B. and Heddle, J.A. (1983). Micronuclei as a rapid and inexpensive measure of radiation-induced chromosomal aberrations. In: Radiation-Induced Chromosome Damage in Man. Alan R. Liss, New York, pp. 93-109.

Krueger, D.E., Milton, R. and Maunder, L. (1980). The Framingham Eye Study: Introduction to the monograph. Surv. Ophthalmol. 24:614-620.

Kuszak, J.R. and Brown, H.G. (1991). Embryology and anatomy of the crystalline lens. In: Principles and Practice of Ophthalmology: The Harvard System, Eds. Albert, D. and Jakobiec, F., W.B. Saunders Press, Phila.

Lagakos, S.W. (1988) Effects of mismodelling and mismeasuring explanatory variables on tests of their association with response variable. Stat. Med. 7:257-274.

Leibowitz, H.M., Krueger, D., Maunder, L., Milton, R., Kini, M., Kahn, H., Nickerson, R., Pool, J. and Colton, T. (1980). The Framingham Eye Study monograph: an ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults. Surv. Ophthalmol. 24:335-610.

Leske, M., Chylack, L. and Wu, S. (1991) The lens opacities case-control study. Risk factors for cataract. Arch. Ophthalmol. 109:244-251.

Lewis, R.A. (1990). Mapping the gene for X-linked cataracts and microcornea with facial, dental, and skeletal features to Xp22: an appraisal of the Nance-Horan Syndrome. Trans. Am. Ophthalmol. Soc. 87:658-728.

Lubin, J.H. and Gail, M. (1984) Biased selection of controls for case-control analyses of cohort studies. Biometrics 40:63-75.

Manski, W., York, A.T., Merriam, G.R., Jr., Worgul, B.V. and Szechter, A. (1976). On the independent action of cataractogenic agents. X-rays and galactose. In: Progress of Lens Biochemistry Research, Doc. Ophthal. Proc. Ser., O. Hockwin, Dr.., Junk Pub., The Hague, Vol. 8, pp. 275-292.

Medvedovsky, C. and Worgul, B.V. (1991) Neutron effects on the lens. Rad. Res. 128:S103-S110.

Merriam, G.R., Jr. and Worgul, B.V. (1983). Experimental radiation cataract - its clinical relevance. Bull. N.Y. Acad. Med. 59:372-392.

Merriam, G.R., Szechter, A. and Focht, E. (1972). The effects of ionizing radiations on the eye. Front Radiat. Ther. Oncol. 6:346-385.

Merriam, G.R., Jr. and Focht, E. (1957). A clinical study of radiation cataracts and the relationship to dose. Am. J. Roentgenol. Radium Ther. Nucl. Med. 77:759-785.

Miller, R.J., Funijo, T. and Nefzger, M. (1967). Lens findings in atomic bomb survivors: A review of major ophthalmic surveys at the Atomic Bomb Casualty Commission (1949-1962). Arch. Ophthalmol 78:697-704.

Muller, L., Kasper, P. and Madle, S. (1991). The quality of genotoxicity testing of drugs. Experiences of a regulatory agency with new and old compounds. Mutagenesis 6(2):143-149.

Munoz, B., Tajchman, U., Bochow, T. and West, S. (1993). Alcohol use and risk of posterior subcapsular cataracts. Arch. Ophthal. 111:110-112.

Murray, D., Prager, A., Altschuler, E.M. and Brock, W.A. (1991). Effect of thiols on micronucleus frequency in γ-irradiated mammalian cells. Mut. Res. 247:167-173.

Nam, J. (1987). A simple approximation for calculating sample sizes for detecting linear trend in proportions. Biometrics 43:701-705.

National Cancer Institute (1989). Health Habits and History Questionnaire: Diet History and Other Risk Factors - Personal Computer System Packet.

Nordmann, J. and Mandel, P. (1955) Enzyme studies in radiation cataract. Am. J. Ophthal. 40:871-880.

Odrich, S., Medvedovsky C., Merriam, G.R., Jr., and Worgul, B.V. (1988). Micronucleation in the lens epithelium following *in vivo* exposure to physical and chemical mutagens. Lens Res. 5(3-4):203-216.

Okada, S. (1970). Radiation Biochemistry. In: Cells, Vol. 1, Academic Press, New York.

Omelyanets, N.I., Misetskij, G.I., Saurov, M.M. and Tabin, V.F. (1988). Medical demographic consequences of the Chernobyl accident In: Medical Aspects of the Chernobyl Accident. Report-IAEA-TEDOC-516, pp. 315-326.

Otake, M., Finch, S., Chosi, K., Takaku, I., Mishima, H. and Takase, T. (1992). Radiation related ophthalmological changes and aging among Hiroshima and Nagasaki A-bomb survivors: a reanalysis. Radiat. Res. 131:315-324.

Otake, M. and Schull, W. (1990). Radiation related posterior lenticular opacities in Hiroshima and Nagasaki atomic bomb survivors based on the DS86 dosimetry system. Radiat. Res. 121:3-13.

Otake, M. and Schull, W. (1982). The relationship of gamma and neutron radiation to posterior lenticular opacities among atomic bomb survivors in Hiroshima and Nagasaki. Radiat. Res. 92:574-595.

Podgor, M.J., Leska, M. and Ederer, F. (1983). Incidence estimates for lens changes, macular changes, open angle glaucoma and diabetic retinopathy. Am. J. Epidemiol. 118:206-212.

Ramalho, A., Sunjevaric, I. and Natarajan, A.T. (1988). Use of the frequencies of micronuclei as quantitative indicators of X-ray induced chromosomal aberrations in human peripheral blood lymphocytes: comparison of two methods. Mut. Res. 207:141-146.

Ritter, L., Klein, B., Klein, R. and Mares-Perlman, J. (1993) Alcohol use and lens opacities in the Beaver Dam Eye Study. Arch. Ophthalmol. 111:113-117.

Robins, J., Gail, M. and Lubin J. (1986) More on biased selection of controls for case-control analyses of cohort studies. Biometrics 42:293-299.

Rothstein, H. (1968). Experimental techniques for the investigation of the amphibian lens epithelium. In: Methods in Cell Physiology, III, Prescott, D.M., Ed., pp. 45-74, Academic Press, New York.

Rothstein, H., Worgul, B.V., Medvedovsky, C. and Merriam, G.R., Jr. (1982). G0/G2 arrest of cell proliferation in the ocular lens prevents radiation cataract development. Ophthal. Res. 14:215-220.

Rothstein, H., Worgul, B.V. and Weinsieder, A. (1982). Regulation of lens morphogenesis and cataract pathogenesis by pituitary dependent, insulin-like, mitogens. In: Cellular Communication During Ocular Development, eds. Sheffield, J. and Hilfer, S., Springer-Verlag, New York.

Samuels, B. (1947). Proliferation of the epithelium of the lens. Am. J. Ophthalmol. 30:1-11.

Santos-Mello, R. and Cavalcante, B. (1992). Cytogenetic studies on gas station attendants. Mut. Res. 280:285-290.

Santo, F., Tomanin, R., Giacomelli, L., Canova, A., Raimondi, F., Ghiotto, C. and Fiorentino, M.V. (1990). Evaluation of chromosomal aberrations in lymphocytes and micronuclei in lymphocytes, oral mucosa and hair root cells of patients under antiblastic therapy. Mut. Res. 228:157-169

Savage, J.R.K. (1989). A comment on the quantitative relationship between micronuclei and chromosomal aberrations. Mut. Res. 207:33-36.

Scarfi, M.R., Cossarizza, A., Monti, D., Bersani, F., Zannotti, M., Lioi, M.B. and Franceschi, C. (1990). Age-related increase of mitomycin C-induced micronuclei in lymphocytes from Down's syndrome subjects. Mut. Res. 237:247-252.

Schimizu, Y., Kato, H. and Schull, W. (1990). Studies of the mortality of A-bomb survivors. 9. Mortality, 1950-1985: Part 2, cancer mortality based on the recently revised doses (DS86). Radiat. Res. 121:120-141.

Schmid, W. (1976). The micronucleus test for cytogenic analysis. In: Chemical Mutagesn, A. Hollander, Plenum Press, New York, Vol. 4, pp. 31-43.

Sergeev, G.V. (1988). Medical and sanitary measures taken to deal with the consequences of the Chernobyl accident. In: Medical Aspects of the Chernobyl Accident. Report - IAEA-TEDOC-516, pp. 23-38.

Shlaifer, A. (1954). Cataract and its etiology. Am. J. Optometry 31:437-458.

Sperduto, R.D. and Hiller, R. (1984). The prevalence of nuclear, cortical and posterior subcapsular lens opacities in a general population sample. Ophthal. 91:815-818.

Stark, W.J., Sommer, A., and Smith, R.E. (1989) Changing trends in intraocular lens implantation. Arch. Ophthal. 107:1441-1444.

Stevens, W., Till, J., Thomas, D., Lyon, J., Kerber, R., Preston-Martin, S., Simon, S., Rallison, M. and Lloyd, R. (1992). Report of a cohort study of thyroid disease and radioactive fallout from the Nevada Test Site. University of Utah, report to the National Cancer Institute, Salt Lake City, Utah (submitted for publication).

Straume, T., Lucas, J.N., Tucker, J.D., Bigbee, W.L. and Langlois, R.G. (1992) Biodosimetry for a radiation worker using multiple assays. Health Physics 62:122-130.

Streeten, B.W. and Eshaghian, J. (1978). Human posterior subcapsular cataract. Arch. Ophthal. 96:1653-1658.

Sutou, S. and Sato, S. (1990) Maternal effect on micronucleus induction in MS/Ae mice. 15:125-130.

Tamura, R.N., Garriott, M.L. and Parton, J.W. (1990). Pooled inference across sexes for the *in vivo* micronucleus assay. Mut. Res. 240:127-133.

Tao, F., Medvedovsky, C., David, J., Broglio, T., Powers-Risius, P., Alpen, E.L. and Worgul, B.V. (In Press) Accelerated heavy ions and the lens: IX The late effect of LET and dose on cellular parameters in the murine lens. Int. J. Rad. Biol.

Thompson, D., Mabuchi, K., Ron, E., Soda, M., Tokunaga, M., Ochikubo, S., Sugimoto, S., Ikeda, T. and Terasaki, M. (1993). Solid tumor incidence in A-bomb survivors, 1958-1987. Radiat. Res. (In Press).

Tomanin, R., Ballarin, C., Nardini, B., Mastrangelo, G. and Sarto, F. (1991). Influence of smoking habit on the frequency of micronuclei in human lymphocytes by the cytokinesis block method. Mutagenesis 6:123-126.

Urlando, C. and Heddle, J.A. (1990). On the differential responsiveness of males and females in the micronucleus assay. Mut. Res. 234:199-204.

van Hummelen, P. and Kirsch-Volders, M. (1992). Analysis of eight known or suspected aneugens by the *in vitro* human lymphocyte micronucleus test. Mutagenesis 7:447-455.

von Sallmann, L. (1950). Experimental studies on early lens changes after X-ray irradiation. Tr. Am. Ophthal. Soc. 48:228-242.

von Sallmann, L. (1951a). Experimental studies on early lens changes after Roentgen irradiation. Arch. Ophthal. 45:149-164.

von Sallmann, L. (1951b). Experimental studies on early lens changes after Roentgen irradiation. III. Effect of X-radiation on mitotic activity and nuclear fragmentation of lens epithelium in normal and cysteine treated rabbits. Arch. Ophthal. 47:305-320.

von Sallmann, L. (1952). The effects of radiation on the cytology of the eye. J. Cell Comp. Physiol. 39:217-233.

von Sallmann, L. (1957). The lens epithelium in the pathogenesis of cataract. The XIII Edward Jackson Memorial Lecture. Am. J. Ophthal. 44:159-170.

von Sallmann, L. (1965). Cytologic studies on lens epithelium: A comparison of effects of X-rays, Myleran and TEM. Invest. Ophth. 4:471-479.

von Sallmann, L., Casavaggio, L., Munoz, C.M. and Drungio, A. (1957). Species differences in the radiosensitivity of the lens. Am. J. Ophthal. 43:693-704.

von Sallmann, L., Dische, Z., Ehrlich, G. and Munoz, C.M. (1951). Study on penetration of cysteine and cystine into the aqueous humor of rabbits and its relation to early X-irradiation effects on the eye. Am. J. Ophthal. 34:95-103.

von Sallmann, L. and Grimes, P. (1966). Some effects of anticancer drugs on cell proliferation in the eye: an experimental study. Ophthalmologica Addit. 151:917-920.

von Sallmann, L. and Grimes, P. (1966). The effect of triethylene melamine on DNA synthesis and mitosis in the lens epithelium. Docum. Ophthal. 20:1-12.

von Sallmann, L., Halvedr, J.E., Collins, E. and Grimes, P. (1966). Thioacetamine-induced cataract with invasive proliferation of the lens epithelium in rainbow trout. Cancer Res. 26:1819-1825.

Wakata, A. and Susaki, M.S. (1987). Measurement of micronuclei by cytokinesis block method in cultured Chinese hamster cells: comparisons with types and rates of chromosome aberration. Mut. Res. 190:51-57.

Weinberg, R.A. (1985). The action of oncogenes in the cytoplasm and nucleus. Science 230:770-777.

Worgul, B.V. (1982). The Lens. In: Biomedical Foundations of Ophthalmology, Vol. I, Chapter 15, pp.1-35, eds., Duane, T. and Jakobiec, F., J.B. Lippincott, Co., Philadelphia.

Worgul, B.V., Brenner, D.J., Medvedovsky, C., Merriam, G.R., Jr. and Huang, Y. (1993) Accelerated heavy particles and the lens VII: The cataractogenic potential of 450 MeV/amu iron ions. Invest. Ophthal. Vis. Sci. 34:184-193.

Worgul, B.V., David, J., Odrich, S., Merriam, G.R., Jr., Medvedovsky, C., Merriam, J.C., Trokel, S.L. and Geard, C.R. (1991). Evidence of genotoxic damage in human cataractous lenses. Mutagenesis 6(6):495-499.

Worgul, B.V., David, J., Tao, F., Medvedovsky, C.M., Burke, P., Merriam, J.C., Merriam, G.R. and Geard, C. (1993) Genotoxic damage and cataracts: gone FISHing. Invest. Ophthal. Vis. Sci..

Worgul, B.V., Medvedovsky, C.M. and Merriam, G.R. (1989). Cortical cataract development - the expression of primary damage to the lens epithelium Lens & Eye Tox. Res. 6: 559-571.

Worgul, B.V., Merriam, G.R., Jr., Szechter, A. and Srinivasan, B.D. (1976). The lens epithelium and radiation cataract I. Preliminary studies. Arch. Ophthal. 94:996-999.

Worgul, B.V., Merriam, G.R., Jr., Medvedovsky, C. and Brenner, D.J. (1989). Accelerated heavy particles and the lens III. Cataract enhancement by dose fractionation. Rad. Res. 118:93-100.

Worgul, B.V. and Rothstein, H. (1974). On the mechanism of thyroid mediated mitogenesis in adult anura. I. Preliminary analyses of growth kinetics and macromolecular synthesis, in lens epithelium, under the influence of exogenous triiodothyronine. Cell and Tissue Kinetics 7:415-425.

Worgul, B.V. and Rothstein, H. (1975). Radiation cataract and mitosis. Ophthal. Res. 7:21-32.

Worgul, B.V. and Rothstein, H. (1977). On the mechanism of radiocataractogenesis. Medikon 6:5-14.

Wuttke, K., Streffer, C. and Muller, W. (1993) Radiation induced micronuclei in subpopulations of human lymphocytes. Mut. Res. 286:181-188.

Yamamoto, K.I. and Kikuchi, Y. (1980). A comparison of diameters of micronuclei induced by clastogens and by spindle poisons. Mut. Res. 71:127-131.

Zar, J.H. (1984) Biostatistical Analysis (Second Edition). Prentice-Hall, Englewood Cliffs, NJ, 1984.

EYE EXAMINATION

VISIT #	VISIT DATE	INVESTIGATOR	PATIENT NAME	CODE

HISTORY: Before 1986 (eye disease, surgery, etc.)

After 1986 (same, in more detail)

	RIGHT EYE	LEFT EYE
Complaints:		
Dryness	NO ☐ YES ☐	NO ☐ YES ☐
Tearing	NO ☐ YES ☐	NO ☐ YES ☐
Discharge	NO ☐ YES ☐	NO ☐ YES ☐
Pain (localization)		
Ocular	NO ☐ YES ☐	NO ☐ YES ☐
Periocular	NO ☐ YES ☐	NO ☐ YES ☐
Spots		
Shifts with eye movement	NO ☐ YES ☐	NO ☐ YES ☐
Floating	NO ☐ YES ☐	NO ☐ YES ☐
Reduced Vision		
Central	NO ☐ YES ☐	NO ☐ YES ☐
Peripheral	NO ☐ YES ☐	NO ☐ YES ☐
Other complaints (specify)	NO ☐ YES ☐	NO ☐ YES ☐
________________		
Visual Acuity		
Normal	NO ☐ YES ☐	NO ☐ YES ☐
Without Glasses	NO ☐ YES ☐	NO ☐ YES ☐
< 0.1	NO ☐ YES ☐	NO ☐ YES ☐
0.1 - 0.5	NO ☐ YES ☐	NO ☐ YES ☐
> 0.5	NO ☐ YES ☐	NO ☐ YES ☐
With Glasses (specify)	NO ☐ YES ☐	NO ☐ YES ☐
R.E.________________		
________________		
L.E.________________		
________________		
< 0.1	NO ☐ YES ☐	NO ☐ YES ☐
0.1 - 0.5	NO ☐ YES ☐	NO ☐ YES ☐
> 0.5	NO ☐ YES ☐	NO ☐ YES ☐

	RIGHT EYE	LEFT EYE
Refraction:		
Myopia	NO ☐ YES ☐	NO ☐ YES ☐
> 6.0 D	NO ☐ YES ☐	NO ☐ YES ☐
Hypermetropia	NO ☐ YES ☐	NO ☐ YES ☐
Astigmatism	NO ☐ YES ☐	NO ☐ YES ☐
Presbyopia		
Visual Field		
Normal	NO ☐ YES ☐	NO ☐ YES ☐
If NO use diagram page		
Intraocular Tension (specify tonometer) ____________________		
< 20 gr	NO ☐ YES ☐	NO ☐ YES ☐
20 - 24 gr	NO ☐ YES ☐	NO ☐ YES ☐
25 - 30 gr	NO ☐ YES ☐	NO ☐ YES ☐
> 30 gr	NO ☐ YES ☐	NO ☐ YES ☐
Eyelashes		
Normal	NO ☐ YES ☐	NO ☐ YES ☐
If NO ____________________		
Eyelids		
Normal	NO ☐ YES ☐	NO ☐ YES ☐
If NO ____________________		
Lacrimal Structure		
Normal	NO ☐ YES ☐	NO ☐ YES ☐
If NO ____________________		
Conjunctiva		
Normal	NO ☐ YES ☐	NO ☐ YES ☐
Injected	NO ☐ YES ☐	NO ☐ YES ☐
Folliculosis	NO ☐ YES ☐	NO ☐ YES ☐
Pingueculum	NO ☐ YES ☐	NO ☐ YES ☐
Telangiectasis	NO ☐ YES ☐	NO ☐ YES ☐
Other (pericorneal injection, etc.)	NO ☐ YES ☐	NO ☐ YES ☐
Anterior Chamber		
Normal	NO ☐ YES ☐	NO ☐ YES ☐
If NO ____________________		

	RIGHT EYE		LEFT EYE	
Cornea				
Normal	NO ☐	YES ☐	NO ☐	YES ☐
Keratitis	NO ☐	YES ☐	NO ☐	YES ☐
Opacification	NO ☐	YES ☐	NO ☐	YES ☐
Precipitates	NO ☐	YES ☐	NO ☐	YES ☐
Other ______________	NO ☐	YES ☐	NO ☐	YES ☐
______________				
Eye Movement				
Normal	NO ☐	YES ☐	NO ☐	YES ☐
If NO ______________				
______________				
Iris				
Normal	NO ☐	YES ☐	NO ☐	YES ☐
Synechia	NO ☐	YES ☐	NO ☐	YES ☐
Other ______________	NO ☐	YES ☐	NO ☐	YES ☐
______________				
Pupil				
Normal	NO ☐	YES ☐	NO ☐	YES ☐
Mydriatic	NO ☐	YES ☐	NO ☐	YES ☐
Anisocoria	NO ☐	YES ☐	NO ☐	YES ☐
Normal Pupillary Reflex	NO ☐	YES ☐	NO ☐	YES ☐
Lens (see separate form)				
Vitreous Body				
Normal	NO ☐	YES ☐	NO ☐	YES ☐
Structural changes (opacif., etc.)	NO ☐	YES ☐	NO ☐	YES ☐
______________				
______________				
Fundus				
Normal	NO ☐	YES ☐	NO ☐	YES ☐
If NO ______________				
______________				
Optic Nerve	NO ☐	YES ☐	NO ☐	YES ☐
Macula	NO ☐	YES ☐	NO ☐	YES ☐
Vessels	NO ☐	YES ☐	NO ☐	YES ☐
Other ______________	NO ☐	YES ☐	NO ☐	YES ☐
______________				

CONCLUSION:			
	Is it possible to dilate the pupil?	NO ☐	YES ☐
	Is it possible to perform biomicroscopy?	NO ☐	YES ☐
	Is it possible to image the anterior segment?	NO ☐	YES ☐

Signature of Principal Investigator	*Date*

FORM UACOS93-EP

LENS EXAMINATION

(Biomicroscopy with dilated pupil>5 mm)

VISIT #	VISIT DATE	INVESTIGATOR	PATIENT NAME	CODE

LENS CHANGES	RIGHT EYE		LEFT EYE	
CLEAR	NO ☐	YES ☐	NO ☐	YES ☐
Early Changes	NO ☐	YES ☐	NO ☐	YES ☐
1. Polychromatic Sheen in Post. Cap. plane	NO ☐	YES ☐	NO ☐	YES ☐
2. Individual dots	NO ☐	YES ☐	NO ☐	YES ☐
in cortex (fewer than 10):				
Anteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Posteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Equatorial	NO ☐	YES ☐	NO ☐	YES ☐
Supranuclear	NO ☐	YES ☐	NO ☐	YES ☐
3. Individual vacuoles (fewer than 5):				
Anteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Posteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Equatorial	NO ☐	YES ☐	NO ☐	YES ☐
Supranuclear	NO ☐	YES ☐	NO ☐	YES ☐
STAGE 1:	NO ☐	YES ☐	NO ☐	YES ☐
Small spot seen with retroillumination.	NO ☐	YES ☐	NO ☐	YES ☐
Small spot (seen with a slit lamp):				
Anteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Posteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Equatorial	NO ☐	YES ☐	NO ☐	YES ☐
Supranuclear	NO ☐	YES ☐	NO ☐	YES ☐
Nuclear	NO ☐	YES ☐	NO ☐	YES ☐
Aggregate of dots (>10) and/or vacuoles (>5); granulated opacities, cortical spots, waterclefts (seen with a slit lamp):				
Anteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Posteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Equatorial	NO ☐	YES ☐	NO ☐	YES ☐
Supranuclear	NO ☐	YES ☐	NO ☐	YES ☐
Nuclear	NO ☐	YES ☐	NO ☐	YES ☐

LENS CHANGES	RIGHT EYE		LEFT EYE	
STAGE 2: More extensive changes collectively occupy the equivalent of 1/8 - 1/4 of the lens cortex. Little interference with ophthalmoscopy and vitreous visualization.	NO ☐	YES ☐	NO ☐	YES ☐
Anteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Posteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Equatorial	NO ☐	YES ☐	NO ☐	YES ☐
Supranuclear	NO ☐	YES ☐	NO ☐	YES ☐
Extensive changes in the nucleus	NO ☐	YES ☐	NO ☐	YES ☐
STAGE 3: Advanced changes. Ophthalmoscopy could be performed only when clear or semi-transparent areas allow visualization of the vitreous and fundus.	NO ☐	YES ☐	NO ☐	YES ☐
Anteriorly	NO ☐	YES ☐	NO ☐	YES ☐
Posteriorly	NO ☐	YES ☐	NO ☐	YES ☐
In Nucleus	NO ☐	YES ☐	NO ☐	YES ☐
STAGE 4: Premature cataract - almost total opacification of the lens. In some areas it is possible to see the nucleus or posterior cortex of the lens.	NO ☐	YES ☐	NO ☐	YES ☐
STAGE 5: Mature cataract - total opacification of the lens.	NO ☐	YES ☐	NO ☐	YES ☐

SUGGESTED ETIOLOGY?

1. Senile or age related changes____________________
2. Congenital or juveline changes____________________
3. Traumatic cataract____________________
4. Cataract associated with intraocular disease:____________________
5. Cataract associated with systemic disorders:____________________
6. Changes caused by noxious agents (ionizing radiation, drug induced, toxic agents, etc.):__________

Signature of Principal Investigator *Date*

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